KING AND TANNER CRAB RESEARCH IN ALASKA: FINAL REPORT

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FORWARD

A federal budget initiative for crab research has been funded by the United States Congress since 1992 to conduct research relevant to management strategies for king *(Paralithodes, Lithodes)* and Tanner/snow crab *(Chionoecetes)* fisheries in the waters off Alaska. The initiative funds cooperative investigations by the Alaska Department of Fish and Game (ADF&G) and National Marine Fisheries Service (NMFS), reflective of the shared responsibilities for crab research and fishery management by the state and federal governments.

This final report is for the two year period July 1, 1999 to June 30, 2001. The grant period, originally conceived to be three years, was shortened to allow for subsequent king and Tanner crab research to fall under the umbrella of an award titled *"Alaska Groundfish Monitoring"*, beginning July 1, 2001.

INTRODUCTION

This is a final report for NOAA Cooperative Agreement NA97FN0129, titled *"King and Tanner Crab Research in Alaska"* for the period July 1, 1999 to June 30, 2001. Details on background, justification, and a long-term research strategy for crab research were provided in the original statement of work (Kruse 1993) and long-term work plans (Kruse 1994, 1996).

The impetus for this research has come from several decades of economic impacts resulting from major changes in abundance of our major crab stocks. Recently, Tanner crabs *(Chionoecetes bairdi)* in the Eastern Bering Sea (BS) declined sharply in abundance to depressed levels. The fishery was not opened in 1997 and 1998, and in March 1999 the stock was deemed overfished by the Secretary of Commerce. On the other hand, after two years (1994 and 1995) of fishery closures the abundance of red king crabs *(Paralithodes camtschaticus)* in Bristol Bay increased from depressed levels due to a relatively strong 1990 year class. Landings of snow crabs *(C. opilio)* declined sharply due to a series of poor year classes. These changes underscore the importance of understanding the interaction of crab stocks with fisheries and natural fluctuations. The long-term strategy for crab research is to investigate crab stock structure, population estimation techniques, biological productivity, and harvest strategies. Largely due to federal assistance, great strides have been made toward understanding crab population dynamics and management implications.

Two statements of work (Kruse 1999 and Kruse 2000), one for each of the past two years, described six projects for this reporting period: (1) crab biometrics; (2) crab management strategies; (3) crab genetics; (4) snow crab life history in 3 component projects; (5) effects of temperature on Tanner crab growth; and (6) reproductive biology of golden king crabs*.* The three component projects in project 4 are: 4A - wind chill effects on snow crabs, 4B – mortality and growth increments for snow crabs, and 4C - snow crab feeding, growth, and energetics. Final reports for all projects are included here as individual reports, each with an executive summary, description of purpose, approach, results, products, and key words. References for all projects are listed at the end of this report.

PROJECT 1: CRAB BIOMETRICS

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Executive Summary

This project funded a new ADF&G Biometrician II and a Fisheries Biologist II. The FY 00 and FY 01 activities included development of expertise in crab biology and population dynamics and familiarization with federal FMP and state regulations. While subject familiarization is an ongoing activity, the initial goal for the biometrician was a thorough review of crab biological reference points (*BRP*) and overfishing definitions. A three-stage catch survey analysis model was used in the computer simulation to determine maximum sustainable yield (*MSY*) producing harvesting rate and *MSY* producing relative spawning biomass for five Bering Sea and Aleutian Islands (BSAI) crab species – red, blue, and golden king crabs; Tanner and snow crabs. As a part of *BRP* investigation, a natural mortality estimator was developed and applied to 1990s BSAI king crab tag-recapture data. A catch survey analysis model will be developed to assess the Aleutian Islands golden king crab stocks during FY 02.

Purpose of Project

Crab biometrics provides necessary biometric inputs for the Bering Sea and Aleutian Islands (BSAI) crab stock assessment and management. This is a new project with a newly recruited Biometrician and a Fishery Biologist. Therefore many milestones involved start-up: (1) recruit and hire the Biometrician and Fishery Biologist; (2) develop expertise in crab biology and population dynamics, crab fisheries, federal crab FMP and state regulations, and agency and industry contacts; (3) assist the crab plan team by contributing to the annual stock assessment and fishery evaluation (SAFE) document and development of the rebuilding plan for the overfished Tanner crab stock in the Eastern Bering Sea; (4) conduct a major review of overfishing definitions and other biological reference points used in the crab fishery management plan, and (5) estimate growth and mortality parameters using observer, survey, and tagging data for several red king crab stocks in the BS/AI area, (6) initiate development of a length-based model of the BS/AI golden king crab stock, (7) conduct an analysis of soak time effects in the St. Matthew Island blue king crab fishery, (8) complete a study of morphometric study of differences in Tanner and snow crabs and their hybrids (9) complete a study of wind chill effects on survival of red king crabs in the Norton Sound fishery, (10) develop a thermal model of cold air effects on king, Tanner, and snow crabs in the BS and Norton Sound, (11) update a shellfish bibliographic database, (12) write a report on the history of crab fisheries in Alaska, and (13) compile survey and fishery databases for crabs in the BS/AI region.

Plausible estimates of population dynamics parameters are required for formulating sound harvesting strategies. These parameters are yet to be determined for a number of BSAI crab stocks. BSAI crab fishery management plans have been established in accordance with the National Standard 1 of the Magnuson-Stevens Fishery Conservation and Management Act. In the National Standard 1, Maximum Sustainable Yield (*MSY*) rules have been established to determine overfishing rates and stock status with respect to overfished levels. When analyses or data are poor, default rules have been established with various biological reference points largely borrowed from groundfish management plans. Most crab stocks of the Bering Sea and Aleutian Islands fall into the data poor category and have been managed with such defaults. Natural mortality (*M*) is used as a surrogate to *MSY* level of fishing mortality (F_{MSY}) in determining limit and target reference points. Thus, estimation of *M* and determination of appropriate BRPs for crab stocks became primary objectives for this project during the last one-and-a half-year period.

Approach

This project funds a full-time ADF&G Biometrician II (Dr. Siddeek) and 9 months for a Fishery Biologist II (Dr. van Tamelen). Published papers, reports, and 1990s tag-recapture data were used to achieve the two primary objectives –review of crab BRPs and *M* estimation. A virtual population analysis (*VPA*) based minimization routine was developed to estimate *M* from tag-recapture data. A three-stage catch survey model was used in the computer simulation of population dynamics of major BSAI crab stocks with known growth and mortality parameters to determine suitable *MSY* exploitation ratio (E_{MSY}) and relative *MSY* producing biomass levels (B_{MSY}/B_{VIRGIN}).

Results, Evaluation and Conclusions

Of the ambitious list of milestones for this project, nearly all were achieved in a satisfactory manner, as described below.

Recruit Staff and Develop Expertise in Crab Biology, Crab Fisheries, Regulations, and Agency and Industry Contacts. Two competent staff members were hired in 2000. Development of expertise in crab fisheries biology and familiarization of federal crab FMP and state regulations, and agency and industry contacts are ongoing activities. As a member of the crab plan team, the biometrician participated in all team activities. A major review of overfishing definitions and other biological reference points used in the crab fishery management plan became his first goal of research. This work is near completion and a report and a paper are being finalized. He also devoted a significant amount of time on developing a natural mortality estimator applicable for crab tag recapture data analysis. This work is extended to include more historical data. As a member of the head office Steller Sea Lion task force, he wrote a chapter on shellfish fisheries (and their impact on Steller Sea Lion).

Assist The Crab Plan Team. As a crab plan team member, Dr. Siddeek reviewed the Bering Sea *C. opilio* and St. Matthew blue king crab rebuilding plans, and the 2000 SAFE document. He participated in the discussion of various crab rebuilding plans, GHL and opilio bycatch issues through a number of teleconferences.

Review of biological reference points (BRP) for Bering Sea and Aleutian Islands crab stocks. For managing BSAI crab stocks, M is used for FMSY and mean mature biomass during 1983-1997 is used for BMSY. However, BRP estimation for crabs has been complicated by the fact that only males are harvested and that recruitment, in many instances, is largely driven by environmental factors rather than density-dependent responses. In this review the shortcomings of direct use of those groundfish based BRPs were addressed and independent methods considering crab specific growth and mortality processes were used to determine BRPs. The following relationship between FMSY and M was established. This is applicable to any fish or shellfish stock under standard fish population dynamics models:

$$F_{MSY} = [\{(R_{MSY}/R_0)(W_{MSY}/W_0)(1-e^{-Z\lambda})\} / \{(B_{MSY}/B_0)(1-e^{-M\lambda})\} - 1]M$$

Where
R = number of recruits, W = mean weight of a crab in the catch, B = biomass, Z = total mortality, λ = maximum life span-age at recruitment. The subscripts *MSY* and *0* refer to those parameters estimated at the MSY and virgin biomass levels, respectively.

The above relationship indicates that F_{MSY} is equal to M only under very restricted conditions. The simulation studies on crab stocks described below showed that F_{MSY} exceeded M most of the time under Beverton and Holt and Ricker stock-recruitment relationships.

Note that from a scatter plot of R and spawning stock biomass, an approximate R_{MSY} / R_0 ratio could be determined. This value with a range of W_{MSY}/W_0 ratios could be used in the above formula to determine an approximate range of F_{MSY} for a given M. It is obvious that the usefulness of this formula depends on the availability of these pieces of information for any stock.

On the other hand, if sufficient information on growth increment; pre-recruit, recruit, post-recruit, and maturity size ranges are available, a catch survey analysis (CSA) type of simulation can be performed to determine various BRPs (e.g., MSY level of harvesting ratio (E_{MSY}) and relative MSY biomass level).

This simulation was performed on six major BSAI crab stocks: Bristol Bay red king crab, St. Matthew Island and Pribilof Island blue king crab, eastern Bering Sea Tanner crab and snow crab, and Aleutian Islands golden king crab, to investigate the trends in MSY level of harvesting ratio (E_{MSY}), B_{MSY}/B_0 ratio, and log of F_{MSY}/M ratio. The combined (male and female) spawning biomass-per-recruit (*SSB/R*) was determined and used in conjunction with Beverton and Holt and Ricker stock-recruit (*S-R*) relationships for various parametric values to explore the trends of these ratios. Different life history behaviors of crabs were incorporated in estimating *SSB/R*. For example, the biennial spawning behavior of blue king crab was accounted for in the *SSB/R* calculation by considering only half of the CSA simulated mature biomass. The terminal molt at maturity of female Tanner and snow crab was easy to account for in the *SSB/R* calculation by disregarding growth of mature females during its mature life period.

The CSA simulations indicated that E_{MSY} and B_{MSY}/B_0 trends depended on the type of *S-R* relationship and accumulation of spawning biomass. Table 1 provides median values of E_{MSY} and B_{MSY}/B_0 ratios for the six crab stocks under plausible mortality and stock-recruitment assumptions. The approximate range of *MSY* producing biomass as a percentage of virgin biomass for various crab stocks are: Bristol Bay red king crab, 35-45%; St. Matthew Island blue king crab, 20-30%; Pribilof blue king crab, 8-15%; Bering Sea Tanner crab, 10-20%; Bering Sea snow crab, 15-30%; and western Aleutian Islands golden king crab, 10-35%. Thus, MSY producing biomass is below half of the virgin biomass for these stocks. On the other hand, there are wider differences among optimum harvesting rates for the two levels of copulation ratio. The optimum harvesting rate ranged 8-20% and 48-93% for the copulation ratios of 1:1 and 1:3, respectively.

BSAI King Crab Natural Mortality Estimation Using Tag-Recapture Data (Dr. Siddeek). Based on rough maximum life span, a constant annual *M* value of 0.2 for red (*Paralithodes camtschaticus*), blue (*P. platypus*), and brown (*Lithodes aequispinus*) king crabs and 0.3 for Tanner (*Chionoecetes bairdi*) and snow (*C. opilio*) crabs have been determined and used as surrogates for F_{MSY} in developing crab fishery management plans. Tag-recapture data were used to determine plausible *M* values for the BSAI king crab stocks. An age-based virtual population analysis *M* estimator as well as a new length based *M* estimator was applied. The analyses were restricted to tag-release-recaptures during the 1990s to determine recent *M* levels. Some data were not good enough to produce reasonable estimates of *M*. For example, the male Bristol Bay red king crab annual *M* estimate was somewhat high (0.54) compared to the life expectancy of this animal. On the other hand, Aleutian Island male golden king crab data produced a reasonable M value of 0.38. The combined sexes of St. Matthew Island blue king crab data also produced a reasonable value of 0.19. The 1950-1980 historical tag-release-recapture data are currently being assembled to apply these estimators to further investigate this important parameter.

Two oral presentations were presented on the current findings, one at the CRAB2001 symposium in January 2001 and the other at the Alaska Chapter Statistical Association Meeting in April 2001. A manuscript (Siddeek et al. 2001) based on 1990s tag-release-recapture data analysis was submitted for the Lowell Wakefield Symposium Proceedings report.

Soak Times and Non-legal Catch (Dr. van Tamelen). Using data from observers, data on catch rates for various soak times were gathered for the Bristol Bay red king crab, Aleutian Island golden king crab, Bering Sea snow crab, and Bering Sea hair crab fisheries. The total catch and percent of sublegal and female crabs was evaluated for various soak times for all fisheries. This study indicated that increasing soak times resulted in minor decreases, if any, in the catch of sublegal and female crabs. Mathematical models that attempt to describe catch for various soak times were poor predictors of catch in commercial fisheries, indicating that other factors are important. A manuscript of this research has been completed and will be published as a Regional Information Report.

Thermal Exposure Research (Dr. van Tamelen). A draft manuscript of research previously done to assess the effects of exposure to sub-freezing aerial conditions on red king crab in Norton Sound has been completed and is being reviewed by the author who initiated the study. The study experimentally exposed crabs for varying amounts of time to ambient conditions during the Norton Sound winter fishery and found that longer exposures resulted in higher mortality. Interestingly, amphipods were attracted to cold-damaged and hastened their demise. Due to difficulties in experimental design and implementation, this will be published as a Regional Information Report rather than in the peer-reviewed literature.

A thermal model predicting body temperatures of Tanner and snow crabs exposed to air has been developed. All potential heat inputs and outputs were identified and modeled using principles of physics and engineering. After considerable trial and error, replicas of snow crabs were made to determine heat transfer coefficients. Replicas were needed to eliminate evaporative cooling as a heat transfer mechanism. Trials comparing predicted to observed body and leg temperatures have been completed and shown that the model predicts temperatures well. Field data on the conditions experienced by crabs in pots and on sorting tables has been completed and will be supplemented with measurements made by observers.

The application of the model to actual fisheries was verified by comparing the deadloss of delivered snow crabs to the severity of the fishing season indicated by the model. Seasons that were determined by the model to more severe had higher deadloss, indicating that the model is useful in predicting mortality on the fishing grounds. This model will allow prediction of lethal and sublethal effects of exposure on discard crabs given readily available climatological data. The model and field data indicate that there are at least three other significant mechanisms of heat loss in addition to "windchill". These include thermal radiation to the sky on clear, cold nights, evaporative cooling, and thermal radiation and conduction to aluminum sort tables. All of these can have effects equal to or greater than "windchill" under conditions encountered in the Bering Sea. Preliminary results of this research have been presented at the Western Society of Naturalists meeting in December and at the Lowell Wakefield Crab Symposium in January, 2001.

Shellfish Literature Database (Dr. van Tamelen). The shellfish literature database has been converted from Papyrus, an MS-DOS based software, to EndNote, an up-to-date windows application that is a highly useful bibliographic database program. The database was reviewed for errors and corrected by a part-time, temporary employee. In addition, various sources for online searching and journals have been gathered and made available to all interested people. Currently, crab researchers have access to up-to-date bibliographic software, online literature searching capabilities, and several online journals relevant to crab research. A special publication describing the bibliographic software and database is in the final stages of publication.

Crab Database Documentation (Dr. van Tamelen). Crab databases have been located in the various regional offices of ADF&G. It is unrealistic to gather all of the data in one central location, so the current goal is to develop a metadatabase that documents the location of various crab related databases, the status of the data, any corrections that have been made to the database, and other relevant information. Thus, if a researcher would like to see what data is available and who to contact for the data, they could consult the metadatabase. This is potentially a large project. A prototype for the metadatabase has been developed, and we expect to further develop the metadatabase in the next contract year. As part of this project, a history of crab research and fisheries will be compiled. The existence of databases, tell us what was done and it should be relatively easy to produce a written history of crab research, fishery history, and available data.

Unmet Milestones. Specific milestones that were proposed but not achieved were initial development of a length-based model of the BS/AI golden king crab stock, completion of a morphometric study of differences in Tanner and snow crabs and their hybrids, and writing a history of crab fisheries in Alaska. Development of a length-based model for the BS/AI golden king crab stock has yet to be started because the BRP review has taken longer to finalize than expected. This goal will be under taken during FY 02. The completion of the morphometric study was an overly ambitious milestone given the limitations of staff time. Documentation of the history of crab fisheries in the state has been postponed pending completion of the metadatabase, which will facilitate assembly of historical information.

Products

The primary products of this project during FY 00 and FY 01 are presentations and publications. The manuscripts are yet to be published. The following is the list of some of the reports and papers prepared during FY 00 and FY 01:

Kruse, G.H., F.C. Funk, H.J. Geiger, K.R. Mabry, H.M. Savikko, and S.M. Siddeek. 2000. Overview of state-managed marine fisheries in the central and western Gulf of Alaska, Aleutian Islands, and Southeastern Bering Sea, with reference to Steller Sea Lions. Regional Information Report 5J00-10, Juneau.

Siddeek, M.S.M. 2000. Marine fisheries resources, fisheries and marine environmental management, coral reefs, and marine parks in the Northwest Indian Ocean. Report of a Regional Workshop on Fisheries Monitoring, Control and Surveillance, Muscat, Sultanate of Oman, 24-28 October 1999. GCP/INT/648/NOR-Field Report C-3, Technical Paper 1: 101-115. FAO, Rome.

Siddeek, M.S.M. 2000 (abstract). Appropriate biological reference points for poorly understood fish stocks. Presented at the 2000 William R. and Lenore Mote International Symposium held in Florida in October-November, 2000.

Siddeek, M.S.M. 2001 (manuscript). Determination of biological reference points for Bering Sea and Aleutian Islands crab stocks. International Shellfish Fisheries Conference, Spain, October 2001.

Siddeek, M.S.M, L. Watson, S. F. Blau, H. Moore. 2001. Estimating Natural Mortality of King Crabs from Tag Recapture Data. Proceedings of Alaska Sea Grant Symposium of Crabs in Cold Water Regions: Biology, management, and Economics.

van Tamelen, P. G. 2001. Effect of pot soak time on crab catch in the Bering Sea and Aleutian Islands. Regional Information Report No. 5J01-XX. Alaska Department of Fish and Game, Division of Commercial Fisheries. Juneau, Alaska.

Key Words

Crabs, biological reference points, natural mortality, catch survey analysis

PROJECT 2: CRAB MANAGEMENT STRATEGIES

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Executive Summary

This project funds an ADF&G biometrician III to conduct quantitative analyses of abundance, biological, and fisheries data for crab stocks. Analyses during FY 00 and FY 01 continued to focus on population estimation, optimal thresholds and harvest rates, rebuilding strategies, and stock and recruitment relationships. Length-based models and computer simulations are primary tools for these analyses. Projects completed during FY 00 and FY 01 include (1) development of a four-stage catch-survey analysis for the Pribilof Islands red and blue king crab populations; (2) the catch-survey analysis for St. Matthew and Pribilof Islands blue king crabs in 1999 and 2000 and Pribilof Islands red king crabs in 2000; (3) the length-based analysis to assess the 1999 and 2000 Bristol Bay red king crab abundances; (4) analysis on spatial recruitment processes of snow crabs in the eastern Bering Sea; (5) estimating rebuilding probabilities of eastern Bering Sea Tanner crabs; (6) reviewing the length-based analysis for Bristol Bay red king crabs from 1993 to 2000 and conducting retrospective analysis; (7) a study on stock assessment and rebuilding strategy for St. Matthew Island blue king crabs; and (8) a study on uncertainty of natural mortality estimates for eastern Bering Sea snow crabs. A project to assist efforts to develop a length-based model to estimate snow crab abundance in the eastern Bering Sea will be continued into FY 02. The findings were reported in eight manuscripts and technical reports.

Purpose

Sound management requires precise estimates of population abundance and quantitative evaluations of alternative management strategies. In Alaska, many crab stocks are assessed annually by trawl or pot surveys, some are assessed irregularly, and some stocks lack assessments. Population estimation models are needed to make best use of multiple years of data on crab size, sex, and reproductive condition. Such models are necessary to evaluate measurement errors in annual surveys and to generate abundance estimates for stocks that are infrequently assessed.

Estimates of biological production parameters are needed to determine optimal management strategies and to calculate fishery yields for the king and Tanner crab fisheries off the coast of Alaska. For most stocks, the common biological and reference points, such as $F_{0.1}$, yield per recruit, optimum yield, and stock-recruit relationships have not been computed. The utility of fishery thresholds and alternative harvest rates have not been thoroughly evaluated either. Two main goals of this project are to improve population abundance estimation and evaluate alternative harvest strategies for crab stocks in Alaska.

Approach

This project funds an ADF&G biometrician III to conduct quantitative analyses of abundance, biological, and fisheries data for crab stocks. Analyses during FY 00 and FY 01 continued to focus on information germane to harvest policy: population estimation, optimal thresholds and harvest rates, rebuilding strategies, and stock and recruitment relationships. Length-based models and computer simulations are primary tools for estimating population abundance, evaluating optimal thresholds and harvest rates, and developing rebuilding strategies. The National Marine Fisheries Service trawl surveys and ADF&G trawl and pot surveys, catch sampling, and observer reports provide data for these analyses.

Results, Evaluation and Conclusions

This project supported the long-term research plan and crab fisheries management in three ways. First, for all major crab stocks we intend to develop estimates of population abundance by modeling available data. For crab stocks with surveys, the models provide estimates of crab abundance that are relatively insensitive to survey measurement errors in any single year. For crab stocks with only fishery performance data, catch-length models provide abundance estimates. Second, because these models embody critical biological parameters specific to a species and stock, they provide a framework within which to evaluate optimal harvest strategies. Finally, optimal harvest strategies developed under this framework can be used to optimally manage crab fisheries.

The goals and objectives for this project have been achieved with exceptions of molting probabilities of mature male Tanner crabs and Adak red king crab projects. Due to unexpected work on developing rebuilding plans for eastern Bering Sea Tanner and snow crabs and St. Matthew Island blue king crabs associated with findings that these stocks are “overfished,” projects on molting probabilities of mature male Tanner crabs and catch-length analysis for the Adak red king crabs have been postponed. The project on Length-based modeling of snow crabs was transferred to Jack Turnock of NMFS with the assistance of the principal investigator.

The findings from this project during this fiscal year have been disseminated to the users and public. First, a series of technical papers and reports documenting the findings have been published or submitted for publication. We also distributed these manuscripts to management agencies. Second, three presentations on our findings were made in two international scientific conferences (the Alaska Sea Grant Symposiums of Spatial Processes and Management of Fish Populations and Crabs in Cold Water Regions: Biology, Management, and Economics). We also reported our findings in the ADF&G and NMFS interagency meetings in December 1999 and 2000, the Board of Fisheries meeting and the NPFMC’s Scientific and Statistical Committee meeting in the spring of 2000. Finally, our findings were disseminated to the public through informal meetings with the public. Specific results for individual tasks are described next.

King Crab Stock Assessments in 1999 and 2000 and Development of 4-stage Catch-survey Analysis. As an annual routine abundance assessment, the length-based analysis was conducted to assess the 1999 and 2000 Bristol Bay red king crab abundances, and the catch-survey analysis was conducted to assess the 1999 and 2000 Pribilof Islands and St. Matthew Island blue king crab abundances and 2000 Pribilof Islands red king crab abundance. To improve the abundance assessment, we developed a 4-stage model to conduct the catch-survey analysis in 2000. The model adds a prerecruit-II stage to the three-stage model and thus provides better assessments of mature crab abundance in terminal years. It is the first time that a model was used to assess Pribilof Islands red king crabs. Two reports on the status of king crab stocks in the eastern Bering Sea in 1999 and 2000 were prepared (Zheng, J., and G.H. Kruse 1999a and Zheng, J., and G.H. Kruse 2000c in the list of written products below).

Spatial Recruitment Processes of Snow Crabs. Work on spatial recruitment processes of snow crabs in the eastern Bering Sea has been completed. The results were presented in the Alaska Sea Grant Symposium of Spatial Processes and Management of Fish Populations in October 1999. A manuscript of the findings was prepared and submitted for publication in the symposium proceedings (Zheng, J., and G.H. Kruse *in press* in the list of written products below).

Rebuilding Probabilities for Eastern Bering Sea Tanner Crabs. The principal investigator assisted the NPFMC's Crab Plan Team to design a rebuilding plan for eastern Bering Sea Tanner crabs. The principal investigator conducted a computer simulation study on rebuilding schedules, as requested by the NPFMC's Scientific and Statistical Committee. This work was revised and incorporated into a draft Environmental Assessment and Regulatory Impact Review and a manuscript was published with the results (Zheng, J., and G.H. Kruse 2000a in the list of written products below).

Retrospective LBA Analysis of Bristol Bay Red King Crabs. Work has been completed for reviewing the length-based analysis for Bristol Bay red king crabs from 1993 to 2000 and conducting retrospective analyses. The purpose of this study was to identify areas for future improvement. The results were presented in the Lowell Wakefield Crab Symposium in January 2001. A manuscript of the findings has been prepared and submitted for publication in the symposium proceedings (Zheng, J., and G.H. Kruse 2001b in the list of written products below).

Stock Assessment for St. Matthew Island Blue King Crabs. This study addressed the stock assessment problems involving massive die-offs. The 1999 stock assessment of the St. Matthew Island blue king crab stock was used as an example to demonstrate our approach for such situations. We first searched for corroborating evidence of an increase in natural mortality and decrease in survey catchability. We then developed a four-stage catch-survey model to assess the stock under different assumptions of natural mortality in 1999. Finally, we used the model to evaluate the consequences of different natural mortality assumptions on the stock by projecting the stock abundance into the near future. The work has been finished, and the results were presented in the Alaska Sea Grant Crab Symposium in January 2001. A manuscript of the findings has been prepared and submitted for publication in the symposium proceedings (Zheng, J., and G.H. Kruse 2001a in the list of written products below).

Rebuilding Strategy for St. Matthew Island Blue King Crabs. The principal investigator evaluated alternative harvest strategies for this stock. The investigator also assisted the NPFMC's Crab Plan Team to design a rebuilding plan for this stock. ADF&G submitted a proposal for the St. Matthew Island blue king crab harvest strategy to the Alaska Board of Fisheries for its meeting in the spring of 2000, and the proposal was adopted. This work was also incorporated into a draft Environmental Assessment and Regulatory Impact Review. A report containing the results of this project was prepared (Zheng, J., and G.H. Kruse 2000d in the list of written products below).

Snow Crab Population Assessments. First, efforts were devoted to assist Jack Turnock of NMFS to develop a length-based model to estimate snow crab abundance in the eastern Bering Sea through compiling and analyzing data and repeatedly reviewing programming and results. Second, a project was initiated to construct catch-survey models with trawl survey and commercial catch data from 1989 to 2000 to estimate natural mortality for the eastern Bering Sea snow crab stock under different assumptions on molting probability of morphometrically mature males, handling mortality rate of bycatch, and survey catchability. The work has been finished, and the results will be presented in the Symposium on Life Histories, Assessment and Management of Crustacean Fisheries in Spain in October 2001. A manuscript of the findings has been prepared and will be submitted for publication in *Fisheries Research* (Zheng, J. 2001 in the list of written products below).

<u>Products</u>

Main products from this project are publications. These include manuscripts and technical reports referred to above and several papers:

Kruse, G.H., L.C. Byrne, F.C. Funk, S.C. Matulich, and J. Zheng. 2000. Analysis of minimum size limit for the red king crab fishery in Bristol Bay, Alaska. North American Journal of Fisheries Management 20: 307-319.

Zheng, J. 2001. Uncertainties of Natural Mortality Estimates for Eastern Bering Sea Snow Crab, *Chionoecetes opilio*. Alaska Department of Fish and Game, Division of Commercial Fisheries. Unpublished manuscript.

Zheng, J., and G.H. Kruse. 1999a. Evaluation of harvest strategies for Tanner crab stocks that exhibit periodic recruitment. Journal of Shellfish Research 18(2): 667-679.

Zheng, J., and G. H. Kruse. 1999b. Stock status of king crab stocks in the eastern Bering Sea in 1999. Alaska Department of Fish and Game, Regional Information Report 5J99-09, Juneau, Alaska.

Zheng. J., and G.H. Kruse. 2000a. Rebuilding probabilities under alternative rebuilding strategies for eastern Bering Sea Tanner crabs. Alaska Fishery Research Bulletin 7: 1-10.

Zheng. J. and G.H. Kruse. 2000b. Recruitment patterns of Alaskan crabs in relation to decadal shifts in climate and physical oceanography. ICES Journal of Marine Science 57: 438-451.

Zheng, J., and G.H. Kruse. 2000c. Status of king crab stocks in the eastern Bering Sea in 2000. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J00-09, Juneau.

Zheng, J., and G.H. Kruse. 2000d. Overview of stock assessment and recommended harvest strategy for St. Matthew Island blue king crabs. Alaska Department of Fish and Game, Regional Information Report 5J00-06, Juneau, Alaska.

Zheng, J., and G. H. Kruse. 2001a. Assessment and management of crab stocks under uncertainty of massive die-offs and rapid changes in survey catchability. Submitted to the Proceedings of Alaska Sea Grant Symposium of Crabs in Cold Water Regions: Biology, Management, and Economics.

Zheng, J., and G. H. Kruse. 2001b. Retrospective length-based analysis of Bristol Bay red king crabs: model evaluation and management implications. Submitted to the Proceedings of Alaska Sea Grant Symposium of Crabs in Cold Water Regions: Biology, Management, and Economics.

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All published papers from this project are routinely filed with NOAA once reprints have been received. Additional copies are available on request.

Key Words

Crabs, length-based model, stock assessment, optimal harvest strategy, rebuilding strategy, recruitment patterns, natural mortality

PROJECT 3: CRAB GENETICS

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Executive Summary

Genetic data can be a useful complement to morphological and demographic data for defining appropriate units of management for harvested species. The goal of this project has been to shift toward more sensitive molecular genetic methods to detect population boundaries of large commercially important crabs in the Gulf of Alaska and Bering Sea. During the course of this project, we developed eight PCR (polymerase change reaction) primer pairs to amplify microsatellite DNA in red king crab to augment previous allozyme data for populations in the Gulf of Alaska and Bering Sea. Some of these PCR primers also amplify microsatellite DNA in related king crab species. In another study, we used allozyme markers to study golden king crab but, unlike red king crab, failed to find subdivided population structure. This apparent lack of subdivision may stem from higher levels of gene flow between populations or from the failure of a limited number of allozyme markers to detect existing structure. Additional samples of Tanner crabs were also examined for allozyme and microsatellite variability. The results extend previous findings of differences between Bering Sea and Bristol Bay populations and between these populations and populations in southeastern Alaska. Finally, we used allozyme, mtDNA, and nuclear DNA markers to examine hybridization between Tanner and snow crabs. These results indicate that hybridization tends to occur chiefly between male Tanner crab and female snow crab. The occurrence of F_2 and backcrossed individuals also indicates that hybrids can be fertile. Our current focus is on red king crab to more closely examine intra-regional and temporal variability with recently developed microsatellite markers.

Purpose of Project

The overall goal of this project has been to use appropriate molecular genetic methods to estimate the genetic stock structure of commercially important crabs in Alaska. These species include red (*Paralithodes camtschaticus*), blue (*P. platypus*) and golden king crabs (*Lithodes aequispinus*), and Tanner (*Chionoecetes bairdi*) and snow crabs (*C. opilio*). This work is a continuation of progress made in understanding the population structure of various species with geographical surveys of allozyme variability. These results indicated a moderate amount of population differentiation among populations of these species. Allozyme population markers, however, may have limited use in detecting small, but important, genetic differences among populations, because only a few allozyme markers were polymorphic in these species. Additional methods, including surveys of microsatellite DNA markers, were added to the study. The high levels of polymorphism and high mutation rates of microsatellite markers are thought to provide deeper insights into genetic population structure in marine species for which the potential for gene flow among populations is large.

Results, Evaluation and Conclusions

This project successfully accomplished the proposed goals, as described for each major species below, and the results and interpretation are highly satisfactory.

Red King Crab

Generally, red king crab have low levels of allozyme variability within and between populations. A previous population survey showed that only two of 42 allozyme encoding loci had high levels of polymorphism that could be used to infer population structure. However, after grouping samples by region, significant frequency differences were found between samples from the Bering Sea, Gulf of Alaska, and southeastern Alaska. Weak, but significant, frequency differences between samples were also found between samples from the Bering Sea. To increase the possibility of detecting population structure, dinucleotide microsatellite loci were developed by colleagues at the University of Washington. Five of these markers were used to screen 400 red king crabs from seven localities extending from southeastern Alaska to Norton Sound. Although significant frequency differences were detected between populations, persistent heterozygote deficiencies impeded the interpretation of these differences. These markers exhibited moderate to excessive "stutter" bands that may have led to inaccurate allele scoring. Alternatively, asymmetric PCR primer failures may have led to heterozygote deficits. Therefore microsatellite DNA loci with tetranucleotide repeats were developed with the hope they would exhibit the same level of polymorphism without stutter bands (Jarne and Lagoda 1996).

Research approach

After commissioning the development of PCR primers for tetranucleotide loci, we received primers in early 2000 for 23 regions of DNA containing repeat sequences. We first examined these sequences for imperfect repeats and for appropriate primer sites. A total of 15 loci were considered to be likely candidates for constructing PCR primers (Table 1). After PCR in an MJ Research thermocycler, amplification fragments were separated by agarose gel electrophoresis in an Applied Biosystems Inc. (ABI) 377 automated sequencer. PCR fragment sizes were estimated with GeneScan (ver. 3.0; ABI, 1998) software with the local Southern-sizing algorithm. Genotyper (ver. 2.1, ABI, 1996) was used to label alleles in the genotypic arrays, which were scored by two researchers. Some primers produced banding patterns with multiple bands that could not be interpreted with a simple genetic model. Other primers apparently did not amplify any DNA fragments. PCR fragments for eight loci met our criteria for use in population studies and were used to develop multiplex protocols (amplification of more than one microsatellite locus in the same PCR amplification cocktail).

Results and Conclusions

Although these microsatellite PCR primers were developed for red king crab, the primers may also be useful for closely related species. We tested for cross amplification in closely related blue and golden king crabs, and more distantly related Tanner crab, large crabs superficially similar to king crabs in the Family Majidae. We also tested for cross amplification in the Aleutian hermit crab (*Pagurus aleuticus*) and the splendid hermit crab (*Labidochirus splendescens*), a species closely related to king crabs and thought to be an evolutionary link between lithodid and hermit (pagurid) crabs (Cunningham *et al*. 1992). All six loci amplified in golden king crab, but only five in blue king crab (Table 2). *Pca104* amplified in the splendid hermit crab, but none of the PCR primers amplified DNA fragments in Aleutian hermit or Tanner crabs.

Allelic frequencies for five polymorphic loci in red king crab were estimated for seven samples extending from southeastern Alaska to Norton Sound (Figure 1). Frequencies are presented as bubble diagrams, in which the size of a bubble is proportional to the magnitude of the allele frequency (Figure 2). The frequency of the most common allele appears as a white circle. A preliminary examination of these frequencies extends the previous results for allozyme data. The two samples from southeastern Alaska appear to be genetically different from the other samples. This difference may reflect isolation by distance or may be due to past ice age separations. The four samples from south central Alaska and Bristol Bay appear to be similar to one another, except for *Pca107*, which showed a small allele-frequency difference between samples from Kachemak Bay and Kodiak Island and Bristol Bay. This difference also appeared in the allozyme dataset. As for allozyme data, allele-frequency shifts at three loci, *Pca101*, *Pca104,* and *Pca107*, indicated that the Norton Sound sample differed genetically from the remaining samples.

The cumulative genetic data indicate that reproductive migration between populations of red king crab is limited, even though individuals of this species have high potentials for passive movement in currents during a short pelagic larval stage and even though adults move considerable distances to reproductive congregations. Other factors may also limit gene flow between populations, including the presence of suitable habitats for recruitment, current assisted recruitment to natal populations, climate-ocean variability, and predation. In any case, these data present sufficient grounds to manage at least regional, and perhaps local, populations as demographically independent units.

Golden King Crab

ADF&G and the National Marine Fisheries Service (NMFS) identified the examination of the genetic stock structure of golden king crab as a high priority in 1992. Population collections were initiated in 1993; however, due to the remoteness and relatively small size (compared to red king crab) of the golden king crab fisheries, suitable collections were difficult to make. We did not get representative samples until 1994-1996. We then examined allozyme variability in these samples and began the development of other molecular genetic markers.

Approach

A total of 300 crabs, including males and females, from Adak Island (1994), Bering Sea (1996), and southeastern Alaska (Frederick Sound/Chatham Strait, 1996) were examined for genetic variability. We used standard allozyme electrophoretic methods (Aebersold et al. 1987) to resolve the products of 38 allozyme-encoding loci and estimated several population statistics from genotypic frequencies. Geographic heterogeneity among collections was evaluated with contingency-table analysis, F_{ST}, and genetic distances.

Results and Conclusions

The gene expression of several allozyme loci in golden king crabs differed from that in red and blue king crabs, as might be expected for comparisons between genera. This species also had low levels of allozyme variability; 5 (13%) of 38 loci were polymorphic, and only one locus, *EST-*

3, was highly variable. Average observed heterozygosities (H_o) were low, ranging from 0.012 (Bering Sea) to 0.014 (Southeast Alaska). Population samples showed a general lack of allele-frequency differences: F_{ST} values did not differ significantly from zero, and none of the contingency-table comparisons among samples was significant (Table 3). Genetic distances between samples were also small, ranging from 0.0176 (Adak Island vs. Bering Sea) to 0.0187 (Adak Island vs. Southeast Alaska). These low levels of within- and between-population allozyme variability are similar to other decapod crustaceans, which also have low levels of allozyme variability (Nelson and Hedgecock 1980).

This survey failed to show a subdivided population structure in golden king crab, as was found with red king crab. However, we are uncertain whether the apparent lack of subdivision stems from greater levels of gene flow between populations or from the failure of a limited number of allozyme markers to detect existing genetic separations among populations. Future studies of genetic population structure in golden king crab may benefit from the development of more variable microsatellite loci.

Tanner Crab

Allozymes

Merkouris et al. (1998) detected allozyme-frequency differences among Tanner crab populations in the Bering Sea, Gulf of Alaska, and Southeast Alaska. Weaker population differences were also detected in the Bering Sea. Allele frequencies in samples collected in Bristol Bay (east of about 163^o 00' W) were significantly different from frequencies in samples near the Pribilof Islands (west of 167^o 00' W). Additional samples were collected in 1999 to examine the structure of populations in Bristol Bay, and these findings are reported here.

Results and Conclusions

Twenty-eight loci were scored in Tanner crab collected in Bristol Bay in 1999. Eight loci were polymorphic; however, only one locus was highly variable. Since genotypic frequencies at all polymorphic loci conformed to Hardy-Weinberg expectations, we assumed this sample was representative of a single panmictic population. Temporal and geographical allele-frequency variability was tested among Bristol Bay samples collected in 1990, 1991, and 1999. No significant differences were found either among years or among areas within Bristol Bay. The Bristol Bay samples were, therefore, pooled and compared to pooled Bering Sea-Pribilof Islands frequencies for 25 loci common to both data sets. As before, allele frequencies differed significantly between Bristol Bay and Bering Sea-Pribilof Islands samples ($P < 0.001$). However, in contrast to previous results, allele frequencies of *IDHP-1* did not differ significantly (P = 0.480) between these two groups following the addition of new Bristol Bay samples.

Since Tanner crab stocks in the Bering Sea are currently depressed, commercial fishing has remained closed since 1996, and a stock re-building plan is now in place. Although crabs in Bristol Bay and the Pribilof Islands are considered to consist of one stock, they have been partitioned into separate groups with individual guideline harvest levels (GHLs) based on geographical differences in local abundances. The results of genetic analyses support this subdivision of Bering Sea stocks. Other biological data also support the subdivision of stocks in the Bering Sea. Otto (1982) reported size-frequency differences between Pribilof Island crabs and those north of the Alaska Peninsula, including Bristol Bay. These data also show differences between Pribilof Island crabs and those along the continental slope of the southeastern Bering Sea. Somerton (1981) also found size differences between large females in the eastern Bering Sea in areas divided by 167^{o} 15' W long. However, since we lack samples west of about 173^{o} W, we are unable to test whether crabs collected near the Pribilof Islands differ genetically from continental-slope crabs.

Microsatellite DNA

To increase the chances of detecting stock differences, we used microsatellite PCR primers developed for snow crabs (*Chionoecetes opilio*) by Urbani et al. (1998) to test for stock differences in Alaskan Tanner crabs. Our work focused on assessing whether these primers could be use in closely related Tanner crabs. We initially screened crabs from the Bering Sea (N=105), Bristol Bay (N=100), and Seymour Canal (Southeast Alaska, N=75). DNA was extracted from 30-100 mgs of frozen muscle tissue using standard extraction protocols (Sambrook et al. 1989). DNA extracts were suspended in TLE buffer and stored at 4^{o}C. For samples requiring re-extraction or re-runs, DNA was isolated using QIAGEN's DNeasy Tissue Kit. Five PCR primers were used to screen for variability in Tanner crabs at five microsatellite loci: *Cop*4-1, *Cop*3-4, *Cop*10, *Cop*24-3, and *Cop*111. PCR amplifications of three loci, *Cop*4-1, *Cop*3-4 and *Cop*111, showed stutter bands or uninterpretable multiple fragment bands and were dropped from further consideration. *Cop*10, consisting of dinucleotide repeats, and *Cop*24-3, consisting of tetranucleotide repeats, were selected for further analysis. Standard methods were used to estimate allelic sizes, to label alleles and to generate genotypic frequencies. Standard statistics were estimated with FSTAT (V. 2.9.1, Goudet 2000), and genetic differentiation between samples was tested with a contingency-table analysis and F statistics.

Results and Conclusions

*Cop*10 and *Cop*24-3 were polymorphic in each population but the degree of polymorphism differed. For *Cop*24-3, the number of alleles ranged from 27 (Bering Sea) to 35 (Bristol Bay), and for *Cop*10 they ranged from 8 (Bering Sea) to 10 (Bristol Bay, Southeast Alaska). Similarly, expected heterozygosities were greater for *Cop*24-3 (H_E = 0.943-0.956) than for *Cop*10 (H_E = 0.559-0.657). This difference in polymorphism between loci was also evident in a comparison of allele frequency distributions (Figure 3). The frequency of the most common allele for *Cop*10 was greater than 0.50 in each population, while the frequency of the most common allele for *Cop*24-3 was less than 0.13. Permutation tests of the statistic $\hat{f}$ showed no statistically significant deviation from Hardy-Weinberg proportions in the three populations. Allele frequencies differed significantly between the Bering Sea sample and the samples from Bristol Bay and southeastern Alaska ($P < 0.003$), but frequencies for the latter two samples did not differ from one another.

Population differences found with these two microsatellite loci are concordant with the partitions seen with allozymes. These results suggest that microsatellites may be useful in complementing allozymes to discern the genetic population structure of Tanner crab. As with other species of large commercial crabs, these results indicate that movement between areas is limited and that regionally defined populations or groups of populations should be the focus of management entities.

Tanner-Snow Crab hybrids

Research examining hybridization and gene introgression between Tanner and snow crab using mtDNA, allozymes, and nuclear DNA (ITS) is now complete. Results of the study, entitled "A genetic investigation of hybridization between *Chionoecetes bairdi* and *C. opilio* in the Bering Sea" were presented orally at the 1999 American Fisheries Society annual meeting and as a poster at the 2001 Lowell Wakefield Symposium. The analysis of nuclear genes and maternally inherited mtDNA genes indicate bi-directional mating between *C. bairdi* and *C. opilio* crabs with mating of male *C. bairdi* with female *C. opilio* crabs predominating. Most of the interspecies individuals that we collected were first generation (F_1) hybrids, but some were of F_2 and backcrossed individuals, providing evidence of hybrid fertility. A manuscript documenting the findings is near completion and will be submitted for review in the near future.

Conclusion and Future Directions

A few trends have emerged from this study that generally show limited dispersal between populations of large commercial crabs. First, small but biologically significant regional differences occur between populations of king and Tanner crabs. These differences are reflected in both allozyme and microsatellite allele frequencies. Second, small-scale genetic differences are also apparent within some regions. For example, frequency differences were found between populations of red king crab and Tanner crab in the Bering Sea. Larger differences were also found between samples of red king crab from Deadman Reach and Barlow Cove populations in southeastern Alaska, even though these populations are only a few tens of kilometers apart. Asynchronous trends in abundance also indicate these two populations may be demographically independent from each other. Third, these latter two populations also had lower levels of genetic diversity. Fourth, some results indicate that frequencies may change in a single population. These changes may be due to sweepstakes recruitment and year-class shifts or our inability to resample the same population with certainty. Our current focus, therefore is to examine with recently developed microsatellite markers intra-regional and temporal variability more closely.

Products

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Key Words

Allozymes, microsatellites DNA, genetic population structure, red king crab, *Paralithodes camtschaticus*, golden king crab, *Lithodes aequispinus*, Tanner crab, *Chionoecetes bairdi*, snow crab, *Chionoecetes opilio*, Tanner-snow crab hybrids, Gulf of Alaska, Bering Sea

Table 1. Microsatellite loci cloned from *Paralithodes camtschaticus*, red king crab.

GIS Locus	ADF&G Locus Identification	Size Range (bp)	Repeat Motif	Annealing Temp. (^oC)	Primer Sequences
KCA008		Multiple bands	$(TAA)^{13}$	56	5'-TTTTGTCTGCATCATATACCG-3' 5'-GCTAACCTAATCCCACAAGG-3'
KCA017	*Pca100*	286-306	$(TAA)^{11}$	56	5'-GGTGCTCATCATTACTCAGG-3 '5'-ACAGAGAAACGGATGAAGG-3'
KCA119		No amplification	$(TTA)^{3}$TTGTTATCA$(TTA)^{3}$TCA$(TTA)^{12}$	56	5'-CCTCCTCCTCTTCTCTTACCA-3 '5'-GGTTATTCCCCTTTCGCTAT-3'
KCA124		Multiple bands	$(ATT)^{17}$	56	5'-GTGTTGAGGTTGCTTCACG-3 '5'-ATCCCACCAATACTCACC-3'
KCA147	*Pca103*	247-274	$(ATT)^{15}(AGT)^{4}$	56	5'-AGAAAGGTCAAGTGTATTAGCC-3' 5'-CAAATACGAGTAAGTTCTTTAGTGC-3'
KCA220	*Pca105*	213-239	$(TAA)^{10}$	56	5'-TAGGAGAGCGGTGTGAGAG-3' 5'-CAGTGGCAAAATAGCCTAAA-3'
KCD048		No amplification	$(TCTA)^{10}$	56	5'-TTGAGTAGGCACCAGTAAGG-3 5'-CATGATGGAGTGTTGAGAGC-3'
KCD116	*Pca101*	231-283	$(TATC)^{18}$AA$(TCAA)^{4}$	56	5'-TTTCGGTTACTCGATATAATGC-3' 5'-TTTTTCTCTGCTTACGAAGG-3'
KCD143	*Pca102*	253	$(TAGA)^{2}$TATA$(TAGA)^{3}$TAAA$(TAGA)^{7}$	56	5'-TCCTCTCGGGAGATAAGG-3 '5'-GTTTTGCACACATTTTCAGC-3'
KCD211a		No amplification	$(TCTA)^{14}$	56	5'-ATAGGGTCTGGCTACAAACTG-3' 5'-CATTAAAGGGGTTACCGAATA-3'
KCD112b		No amplification	Unavailable	56	5'-AAAGGGGTTACCGAATAGGG-3' 5'-CCTCTCTCCTCTTGGCTCTG-3'
KCD219	*Pca104*	190-242	TATCTATATCTGAA$(TATC)^{2}$TAAGCC$(TATC)^{2}$TAGCTAGC $(TATC)^{10}$	56	5'-ACAGACACACATACACTTTCTCC-3' 5'-GTGGGATAACCATGACACC-3'
KCD232		No amplification	$(AGAT)^{5}$AGGTAGATAGAT$(AGAT)^{5}$GGAT$(AGAT)^{11}$	56	5'-AGACACACTGGGAGGGAGTAT-3' 5'-TCTCAACCAGTGCCAAAG-3'

GIS Locus	ADF&G Locus Identification	Size Range (bp)	Repeat Motif	Annealing Temp. (^oC)	Primer Sequences
KCD269	*Pca106*	131	$(AGAT)^7$AGAGAGAT$(AGAT)^2$	56	5'-AAATAAATAAATAAGGGGTATGTGC-3' 5'-TTTCATCCTCAGGTGTTGG-3'
KCD274	*Pca107*	204-276	$(CTAT)^{11}$TT$(CTAT)^6$CTCCCAT$(CTAT)^4$ATATCTATCGGTAT $(CTAT)^{12}$AT$(CTAT)^3$	56	5'-ACCTCTCGTTGTAACTGTGC-3' 5'-TACACCTTGCTGTTCAGTCC-3'

Table 2. Cross-species testing of red king crab primers. Fragment sizes and number of alleles in parentheses. Primers failed to amplify PCR products for Tanner crab (*Chionoecetes bairdi*, *N* = 96) and Aleutian hermit crab (*Pagurus aleuticus*, *N* = 7).

Locus	*L. aequispinus* *N* = 96	*P. platypus* *N* = 96	*Labidochirus splendescens* *N* = 2
Pca100	280-296 (4)	273-289 (5)	-
Pca101	226-242 (5)	222-230 (3)	-
Pca103	249-312 (22)	-	-
Pca104	207-311 (27)	217-333 (27)	103 (1)
Pca105	213, 285 (2)	213 (1)	-
Pca107	204-264 (16)	236-280 (11)	-

Table 3. Allele frequency and observed (H_o) and expected (H_e) heterozygosity estimates for three collections of golden king crabs.

	sAH*			EST-3*			
Population	N	*100	*118	N	*100	*67	*40
Adak Island 1994	100	0.9950	0.0050	92	0.6467	0.2391	0.1141
Bering Sea 1996	100	1.0000	0.0000	94	0.6755	0.2074	0.1170
Southeast AK 1996	100	1.0000	0.0000	96	0.6406	0.2656	0.0938

PEPA-1*	PEPD-1*					
Population	N	*100	*67	N	*100	*131
Adak Island 1994	100	1.0000	0.0000	100	1.0000	0.0000
Bering Sea 1996	97	1.0000	0.0000	100	1.0000	0.0000
Southeast AK 1996	100	0.9950	0.0050	100	0.9950	0.0050

		SOD-3*					
Population	N	*100	*122	*72	*128	*133	*118
Adak Island 1994	100	0.9600	0.0300	0.0000	0.0000	0.0050	0.0050
Bering Sea 1996	100	0.9750	0.0150	0.0100	0.0000	0.0000	0.0000
Southeast AK 1996	100	0.9750	0.0200	0.0000	0.0050	0.0000	0.0000

Population	Ho	He
Adak Island 1994	0.0138	0.0158
Bering Sea 1996	0.0119	0.0142
Southeast AK 1996	0.0144	0.0153

Table 4. Number of alleles (*A*) and expected heterozygosity (H_E) at *Cop*10 and *Cop*24-3 in three population samples of Tanner crab (*C. bairdi*).

Locus		Bristol Bay	Bering Sea	S.E. AK
*Cop*10	A	10	8	10
	H_E	0.638	0.657	0.559
*Cop*24-3	A	35	27	34
	H_E	0.956	0.943	0.951

Figure 1. Locations of samples of red, blue, and golden king crab used for genetic analysis.

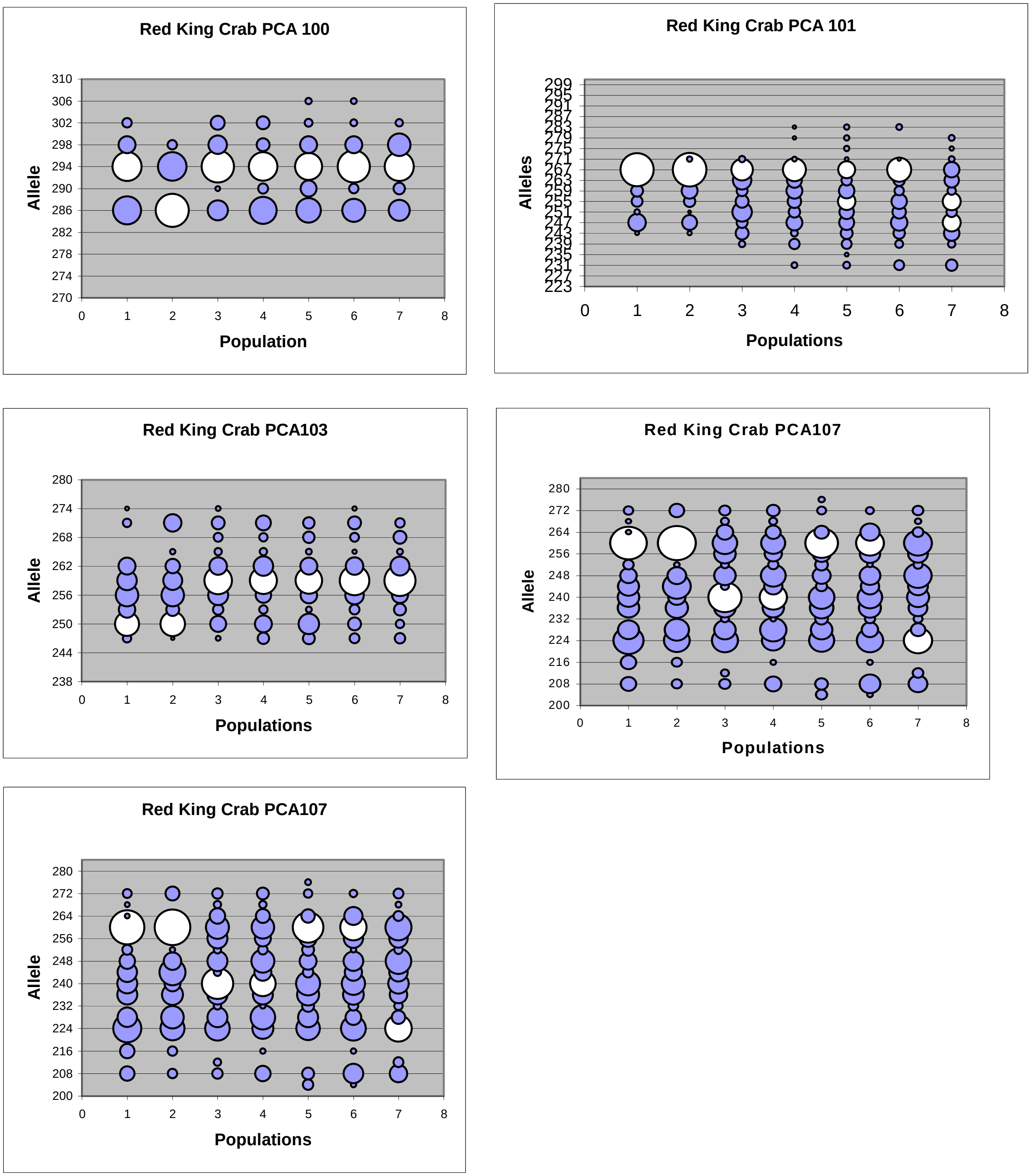

Figure 2. Bubble diagrams of microsatellite allelic distributions and frequencies in red king crab in the Gulf of Alaska and Bering Sea. 1. Barlow Cove; 2. Deadman Reach; 3. Kachemak Bay; 4. Uganik Passage; 5. Pribilof Islands; 6. Bristol Bay; 7. Norton Sound. Size of bubble for each allele is proportional to its frequency. Most frequent allele denoted by clear bubble.

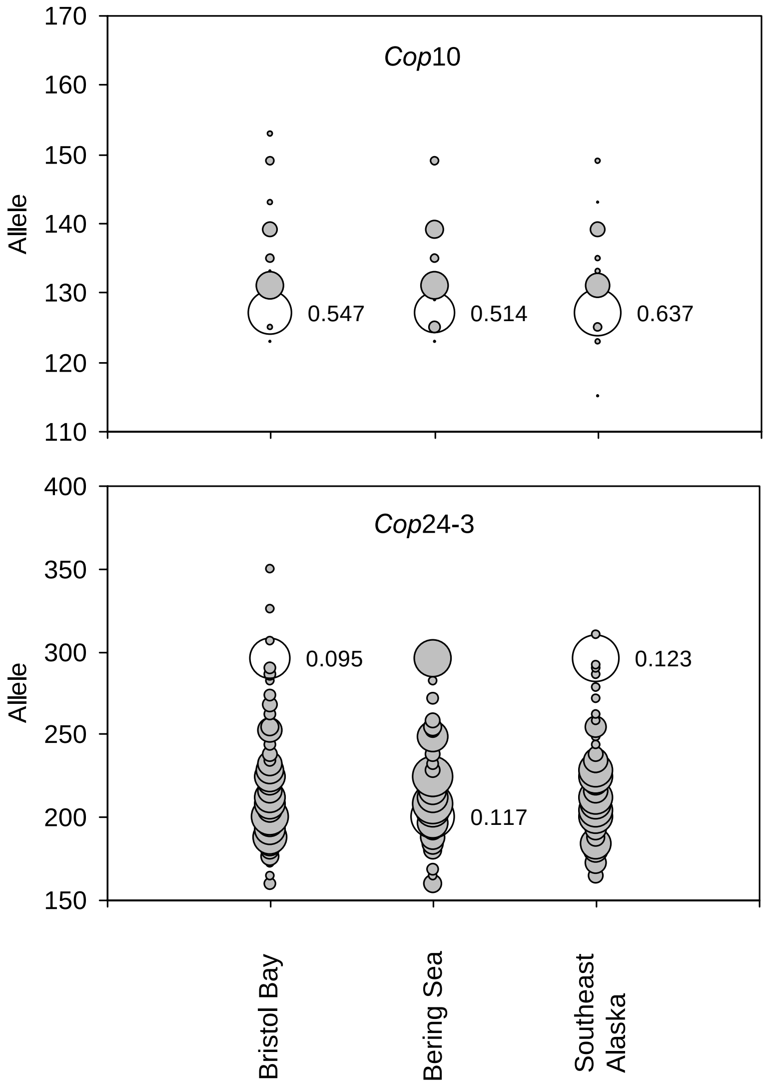

Figure 3. Allele frequency distributions for *Cop*10 and *Cop*24-3 in Bristol Bay, Bering Sea and Southeast Alaska Tanner crab (*C. bairdi*). Circle size is proportional to frequency and the frequency of the most common allele (open circle) is shown for each sample.

PROJECT 4A: EFFECTS OF WIND CHILL ON SNOW CRABS

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Executive Summary

The Bering Sea snow crab fishery occurs during winter and a high probability exists that discarded crabs will be exposed to cold air temperatures and high winds during their aerial exposure. Tens of millions of crabs are sorted and discarded each year. Understanding lethal and sublethal effects of windchill is important to management of the fishery. A laboratory experiment was conducted to measure responses of snow crab to a range of air temperatures and windspeed to assess the effects of windchill. Male snow crabs were collected from the Bering Sea whose sizes (carapace width, CW) approximated those typically discarded during the fishery. A wind tunnel in a walk-in freezer simulated the windy and cold conditions on the deck of a commercial Bering Sea crab boat. Windchill treatments were performed with the following combinations of windspeed and temperature for an exposure time of 5 minutes: -2°C and 8 m/s, -2°C and 16 m/s, -6°C and 8 m/s, -5°C and 16 m/s, -10°C and 8 m/s, and a control with no aerial exposure. The two most severe treatments, -5°C and 16 m/s and –10°C and 8 m/s, were also performed with an exposure time of 2.5 minutes. Observations of limb autotomy, mortality, and righting response were made for each numbered crab immediately after crabs were returned to the wet laboratory, again after 24 hours, and then after seven days. A gradient of responses from mild to severe windchill was observed. Mortality occurred in the three harshest treatments; maximum mortality was 100% at a windchill of approximately –16°C. Shorter exposure time significantly reduced, but did not negate mortality. Limb loss occurred in the moderate to severe exposures and varied from extensive (up to 80% of limbs lost) to minimal (no limbs lost). The righting response was impaired after all but the least severe treatment.

Purpose of Project

The objectives of this study were to determine the effects of windchill exposure (cold air temperature and wind) on mortality, limb loss, and activity (measured as a change in the righting response) for male snow crabs (*Chionoecetes opilio*) of the size typically discarded as bycatch.

Background

The snow crab fishery occurs during winter months when conditions in the Bering Sea are the most severe. Males with a CW less than minimum legal size (<78 mm; referred to as "sublegal") and females must be returned to the sea; crabs deemed unmarketable such as very old shell males, injured crabs, and small males (78-101 mm carapace width) are also sorted and discarded. The discarded snow crabs receive aerial exposure to harsh windchill conditions during pot retrieval and sorting.

Aerial exposure is a stress for any aquatic animal. Obviously, exposure to air creates respiration problems; aquatic animals cannot exist out of water for an extended length of time. Aerial exposure during cold temperatures creates added problems of heat loss for a heterothermic animal. Air movement accelerates heat loss, so the effects of wind must also be considered. The accelerated rate of cooling due to the combination of cold temperatures and wind is referred to as windchill (Court 1948).

Aerial exposure alone has minimal effects on snow crab fitness. In an experiment on temperature tolerance of snow crabs, crabs stored in moist air for 4 days at 3°C or 8°C had no mortality (McLeese 1968). Time to 50% mortality was 8.5 days at 3°C and 1.9 days at 13°C (McLeese 1968), demonstrating that snow crabs can survive aerial exposure to cool moist air for short periods with minimal mortality. Sublethal effects from aerial exposure are unknown.

Aerial exposure to cold air temperatures has more pronounced effects on red king crab and Tanner crab. Short duration exposure to low temperatures caused the same effects as longer exposure at higher temperatures (Carls & O'Clair 1990). Severe exposure (long duration at moderate temperatures or short duration at low temperatures) caused death in Tanner crab and red king crab (Carls & O'Clair 1990, 1995). Moderate exposures caused reduced vigor, limb loss, depressed feeding rates, and decreased juvenile growth in Tanner crab (Carls & O'Clair 1995). Exposure to cold air caused reduced vigor and growth, but did not affect hatching success of ovigerous female red king crab (Carls & O'Clair 1990).

Studies which simulated the capture and release processes found that handling alone does not cause significantly higher mortality in red king crabs (Zhou and Shirley 1996, 1995) and Tanner crabs (MacIntosh et al. 1996). Snow crabs, having morphology similar to Tanner crabs, would be expected to have similar responses to handling. However, handling effects in the laboratory are likely to be conservative when compared to handling effects in the field. During the fishery, snow crabs have the potential to fall from 2 to 4 feet on a hard surface as the pots are tipped (Tracy and Byersdorfer 2000), their extremities can be pinched under the pot, and the release of many crabs through small scuppers can cause damage. Additionally, the weight in air of large numbers of crabs in pots causes crushing to crabs on the bottom of the pot.

The combined effect of exposure to wind and cold air temperature, or windchill, could be the most severe stressor on snow crabs. Red king crab (Shirley 1999) and Tanner crab (Shirley 1998) had pronounced responses to windchill exposure. Laboratory experiments with juvenile, male Tanner crabs demonstrated that exposure to windchill values commonly encountered during the fishery resulted in mortality, limb loss, and decreased activity (Shirley 1998). Five-minute exposures to temperatures of -7^{o}C and wind speed of 16 m/s resulted in 90% mortality of Tanner crabs within a week.

Extreme heat loss can be fatal for any organism, and is especially problematic for a heterothermic snow crab. Heat can be lost by radiation, conduction, or convection. Radiative heat loss occurs as an object radiates heat waves into the surrounding environment. Conductive heat loss occurs from the transfer of heat from one object in contact with another. For example, a crab could lose heat to a cold sorting table, or to the air itself. Sorting tables on commercial crab boats vary in design; conductive heat loss is mitigated when flowing water is pumped across the table. Convective heat loss results from the movement of air over a surface. Air movement disturbs the laminar insulating layer of air around an object and serves to draw heat away from that object. Convective air loss is the major avenue of heat loss that results in windchill.

While snow crab bycatch occurs in many fisheries in the Bering Sea and the Aleutian Islands (BSAI), the snow crab fishery itself accounts for most of the bycatch. Between 1994 to 1999, bycatch due to the snow crab fishery has ranged from 40 million to 75 million crabs (Table 1; Witherell 2000). Of lesser importance are the other crab fisheries (king and Tanner crab), groundfish trawls, groundfish fixed gear, and scallop dredging, which account for the rest. Bycatch of snow crabs is decreasing in the groundfish fisheries (but is not a major component of the total bycatch). The majority of snow crab bycatch are small, legal-sized males. There is a difference between legal size and marketable size of snow crab, as crabs of legal size (79 mm CW), but less than marketable size (102 mm CW) are discarded. The legal, sub-marketable sized crabs constitute the largest bycatch component. Female snow crabs are estimated to account for less than 1% of the snow crab bycatch. Sublegal sized male snow crabs (<79 mm carapace width) are estimated to account for 2% or less of the total bycatch (NPMC 2000).

Table 1: Bycatch of *C. opilio* crabs (numbers of crabs) in Bering Sea fisheries, 1994-1999.

Year	Directed crab pot	Groundfish trawl	Groundfish fixed gear	Scallop dredge	Total
1994	53,082,564	12,351,899	130,228	34,866	65,599,557
1995	48,734,000	5,165,555	230,233	0	54,129,788
1996	56,570,785	3,643,612	267,395	104,836	60,586,628
1997	75,005,446	5,276,208	554,103	195,345	81,031,102
1998	51,591,453	4,122,648	549,139	232,911	56,496,151
1999	41,666,447	1,544,747	269,778	150,421	43,631,393

The total bycatch has declined from a high of 81 million crabs in 1997 to 43 million in 1999. However, bycatch as a percentage of the stock of similar-sized male crabs (<4’’ CW) is increasing (Table 2), probably as a result of increased fishing effort to find crabs when stock abundance is low. The trend had been masked in other reports (Witherall 2000) where bycatch had been reported as a percentage of the estimated abundance of both sexes and all size classes of crabs. Since the majority of bycatch are male snow crabs less than 4” CW, percentages should reflect the estimated abundance of crabs of that size.

The Canadian snow crab fishery in the Atlantic has a different management strategy. The legal size is 95 mm CW and it is illegal to discard any legal-sized crabs (except soft-shell) (B. Sainte-Marie, pers. comm.). Mortality of snow crab bycatch from the directed Bering Sea snow crab pot fishery had been estimated at 24%; this estimate was incorporated into the rebuilding plan (NPFMC 2000).

Table 2: Bycatch and estimated abundance of small males (numbers of crabs).

Year	Bycatch total	Estimated abundance of small male opilio (<4")	Bycatch as % of small males
1994	65,599,557	4,282,500,000	1.53
1995	54,129,788	4,086,800,000	1.33
1996	60,586,628	2,700,100,000	2.24
1997	81,031,102	1,490,800,000	5.44
1998	56,496,151	1,014,700,000	5.57
1999	43,631,393	517,000,000	8.44

Sublethal effects such as autotomy, or limb loss, can occur when snow crabs are caught and discarded. Autotomy is the reflexive severance of an appendage and is considered an adaptation to avoid predation. Autotomy is likely the last attempt to escape capture by a predator after other methods have failed (hiding, fleeing) (Juanes and Smith 1995). The direct benefit of autotomy (survival of predation situation) is offset if future costs are incurred. Autotomy can reduce growth increments as more limbs are lost. Skinner (1985) noted the "regenerative load" where the size increase at moult was decreased by the extent of regeneration required. Reduction in growth, foraging efficiency, mating success and increased vulnerability to predation and intraspecific competion are potential future costs of autotomy. Single limb loss was the most common injury observed in decapod populations (Juanes and Smith 1995). Multiple limb loss is relatively rare, but occurs more frequently than predicted by chance alone. Multiple limb loss is associated with adjacent limbs from the same side indicating that multiple limbs could be lost during a single attack event (Smith 1990).

Snow crabs with regenerated limbs are rarely observed in the catches (R. Morrison, ADF&G, pers. comm). In a laboratory study of limb regeneration, no crabs over 90 mm carapace width were observed to regenerate limbs (Miller & Watson 1976). Crabs formed a scar where a limb had been autotomized; the scar was reformed after molting in the laboratory (Miller & Watson 1976). Smaller crabs regenerate limbs, but require at least two molts to regenerate to 74% of their full length (Miller & Watson 1976). Snow crabs of this size constitute a much smaller proportion of the bycatch (<2%). The majority of discarded snow crabs are of the size that is unlikely to regenerate limbs. If limb loss occurs when a snow crab is caught, it is unlikely that crab will regenerate the limb. Since most of the snow crab bycatch consists of males over 90 mm, limb loss, when it occurs, can be considered total and irrevocable. The loss of biomass is cumulative if the crab is captured again in future fisheries. A snow crab with lost limbs is not commercially desirable. Injury rates in the field were highly variable, but averaged 24% of discarded crabs (Tracy and Byersdorder 2000).

Snow crabs may not be functionally impaired by limb loss, otherwise they would invest the energy to regenerate the lost limb. An alternative explanation is that snow crabs of the size caught in the fishery do not regenerate limbs simply because the time and number of molts required is past terminal molt. Another alternative is that snow crabs must invest the energy to grow larger (larger size being a reproductive advantage) and limb regeneration is compromised. Yet another alternative is that limb loss affects survival in some fashion. *Chionoecetes bairdi, Paralithodes camtschaticus,* and *Homarus americanus* had a negative correlation between body size and injury (Juanes and Smith 1995). A negative correlation between body size and injury may indicate that injury reduces survival, fewer crabs with lost limbs survive to grow to a larger size, or more likely, that smaller crabs incur more injury from predation or competitive interactions.

Approach

Experimental design

This was a laboratory experiment, with male snow crab of the size typically discarded by the fishery (102 mm>79mm carapace width) used as the test organisms. Seven experimental treatments and one control, with 15 replicate crabs in each treatment, comprised the experimental design. Crabs were placed in tanks such that one tank was devoted to each treatment and tanks contained equal numbers of crabs. Each treatment contained a sample of crab sizes with minimized variation in size between tanks.

Exposure times reflected actual sorting time measurements from the field. Crabs were exposed to windchill treatments for 5 minutes, similar to the average maximum aerial exposure measured by observers in 1998 (Tracy & Byersdorfer 2000). The total exposure time was reduced by half to 2.5 minutes for two of the most severe treatments. This is analogous to the short period of time when a crab pot is hauled out of the water and hangs away from the boat in full force of the wind while the crew moves the pot into position. A crab would be shielded from direct wind by equipment, crew, and the movement of the vessel.

Wind and cold treatments were selected using the best available weather data reflective of the Bering Sea during the crabbing season. Unfortunately the best weather data is obtained from a National Weather Service buoy that is 400 miles southwest of the fishing grounds. The recorded weather at the buoy and at the fishing grounds was assumed to be similar. The minimum daily temperature averaged during the 1998 season was –8.6°C. The coldest temperature during the season was –10.4°C. The average daily temperature was -1.2°C. During the 1996 and 1997 seasons, the coldest air temperatures were –8.9^{o}C and –7.7°C, respectively. The average windspeed was 9.8 m/s with average gusts to 24.7 m/s in 1998. In 1996, the average daily windspeed was 10.4 m/s with average gusts to 30.3 m/s. Windchill treatments were performed with the following combinations of windspeed and temperature for an exposure time of 5 minutes: -2°C and 8 m/s, -2°C and 16 m/s, -6°C and 8 m/s, -5°C and 16 m/s, -10°C and 8 m/s, and a control with no exposure. The two most severe treatments, -5°C and 16 m/s and –10°C and 8 m/s, were also performed with an exposure time of 2.5 minutes.

The windchill for each treatment was calculated using the National Weather Service formula:

$$Temp_{windchill} = 0.045*((5.27*\sqrt{Windspeed}) + 10.45 - (0.28*Windspeed))*(Temp_{air} - Temp_{initial}) + Temp_{initial}$$

where Windspeed=mean wind speed (km/hr), $Temp_{air}$=ambient temperature (°C), and $Temp_{initial}$=initial temperature of body (°C)

The initial temperature of a crab body was assumed to be the same as the water temperature from which it was removed (~6°C).

Windchill treatments were performed in a large, walk-in freezer. Temperature was recorded every minute and averaged for the duration of the treatment. A squirrel cage blower was used to generate wind speeds through a wind tunnel (44 x 38 x 239 cm) made of wood and plastic sheeting. An electronic anemometer measured wind speeds within the tunnel. To minimize aerial exposure, crabs were moved to the cold room while immersed in seawater. Crabs were placed inside the wind tunnel while confined within mesh cages (43 x 33 x 23 cm having 2.5 x 3.8 cm mesh) to insure a uniformity of exposure. The cages were placed centrally within the tunnel and oriented into the wind such that no wind blockage occurred between crabs. Immediately after exposure, the crabs were placed in seawater then returned to the tanks from which they came in the laboratory.

The righting response is a complex reflex requiring muscular coordination and balance and can be a sensitive measure of well-being of organisms (Shirley and Stickle 1982). The righting response was determined by placing the crabs on their dorsum and measuring the time in seconds (to a maximum of 300 seconds) required for the crabs to right themselves. Each crab served as its own control, as the righting response of individual crabs was measured prior to and after exposure.

Observations of limb autotomy, mortality, and righting response were made for each numbered crab immediately after crabs were returned to the laboratory, again after 24 hours, and then after seven days. Mortality was determined by detection of movement of the scathognathites (gill bailers), pereopods, mandibles, and maxillae. Functional mortality, where the crab remained moribund for extended periods, was noted. Mortality was assessed daily for 7 days post-treatment and dead crabs were removed from the tanks.

This study was the first to use live *C. opilio* from the Bering Sea in a laboratory experiment. Jon Warranchuk assisted with specimen collections from the Bering Sea prior to the opening of the 2000 season. Crabs were kept moist and transported in minimum possible time to the Auke Bay laboratory in insulated containers. Any crabs identified as having bitter crab disease, black mat syndrome, pepper crab, or torch disease (Jadamec et al. 1999), were not retained. Care was taken to minimize handling and exposure to of the crabs to air. Crabs were maintained in flow-through seawater tanks at the Juneau Center, School of Fisheries and Ocean Sciences and NMFS Auke Bay Laboratories. The seawater intakes for the School of Fisheries and National Marine Fisheries Service laboratories are at –30 m in Auke Bay; temperature and salinity variations were within the range recorded for the waters of the Bering Sea. Seawater discharge from the tanks was passed through a freshwater reservoir before being routed to the seawater return line.

The specimens were observed for two weeks prior to initiation of the experiment to ensure the health and uniformity of replicates. Size measurements, previous damage (old injuries), shell condition, and hemolymph screening were performed on all specimens. Carapace length was measured from the notch between the rostral horns to the posterior of the carapace. Carapace width was measured as the greatest distance across the carapace and does not include the spines. Crabs were numbered with Floy tags attached with a plastic cable tie to the merus of the fourth or fifth pereopod. After the completion of the study, the crabs were frozen and taken to the ADF&G tag lab for disposal. Initial windchill treatments were completed by April 13, 2000; however, some additional experiments were completed in the summer.

Lynnette McNutt, Research Technician, University of Alaska Fairbanks assisted with experimental exposures, data collection and care and feeding of crabs. Dr. Adam Moles, National Marine Fisheries Service, assisted with wet laboratory setup and was a considerable asset to screen hemolymph samples for bitter crab disease.

<u>Results</u>

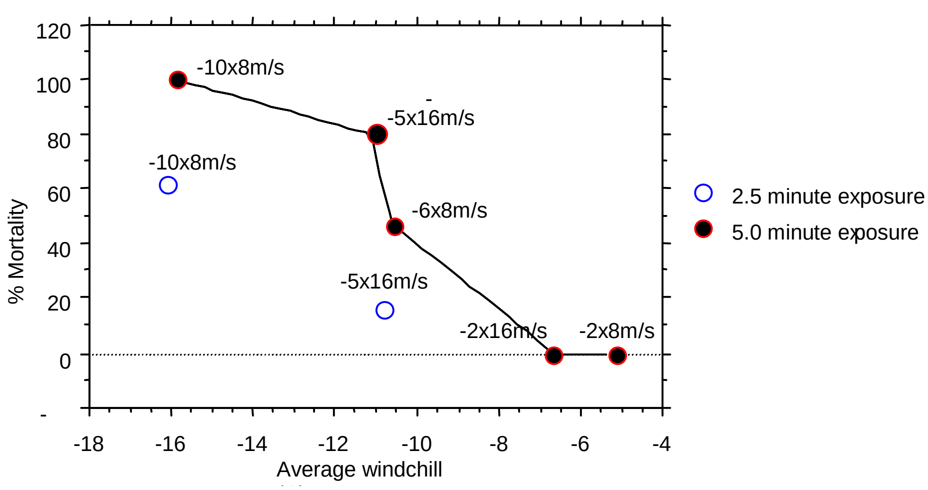
Post-hoc removal of potentially impaired crabs from the data analysis

The snow crabs in the experiment were captured in the wild before being transported to the laboratory for study. It is possible that some crabs were injured or slightly impaired during capture and transportation. Under the assumption that the speed of the righting response is a measure of well being, crabs that had a slow initial righting response were removed from the data analysis. Any crab that did not right within 300 seconds before treatment was removed from data analysis. The removal did not detract from the significance, and only slightly affected the power of the analysis. The removal created unbalanced samples, but robust statistical analysis was still possible.

Mortality

The percentage of crabs that died in each treatment increased with increasing windchill (Fig 1). Mortality was 100% at a calculated windchill of –16 (-10°C and 8 m/s). Mortality was 80% and 40% for the –5°C and 16 m/s and –6°C and 8 m/s, respectively (Fig 1.). No mortality occurred in the two least severe treatments or the control. The percent mortality was significantly different ($p>0.001$) between treatments when mortality occurred. Shorter exposure time reduced mortality (Fig. 1). The mortality for treatments where exposure time was reduced to 2.5 minutes was significantly less ($p>0.001$) than the 5 minute exposure treatments.

Figure 1: Bivariate Scattergram with Lowess,Tension =
Percent mortality in each treatment vs windchill



Autotomy

The percent autotomy for each crab was calculated by dividing the total number of limbs lost after the treatment by the number of limbs the crab had before the treatment. Some of the crabs in the experiment had autotomized limbs previously so not all crabs had 10 limbs initially. Of the 120 specimens used in this study, none had regenerated limbs, and 56% had previous injuries. Autotomy was measured as the proportion of limbs lost. Proportions generally form a binomial rather than normal distribution (Zar 1996). An arcsine transformation did not generate a normal underlying distribution. A square root transformation of the data was used to visually assess trends in a bivariate scattergram (Fig. 2).

A non-parametric Tukey test was used to measure significance of autotomy attributed to the treatments (Table 3). Only the crabs that survived treatments were used to test significance of autotomy. The control group and the exposures of –2°C x 8 m/s and –2°C x 16 m/s were not significantly different from one another. A significant difference existed amongst all other treatments. No significant difference occurred between the moderate and severe windchill treatments (Table 3). High variability was associated with limb loss among windchill treatments. For example, a range of 0 to 80% autotomy was observed at a windchill of –10.5 °C (Fig. 1). Autotomy for the shorter exposure time was not significantly different from the 5 minute exposure.

Figure 2. % limb loss^1/2 vs windchill

Bivariate Scattergram with Lowess smooth curve (Tension = 66)

Inclusion criteria: Surviving crabs

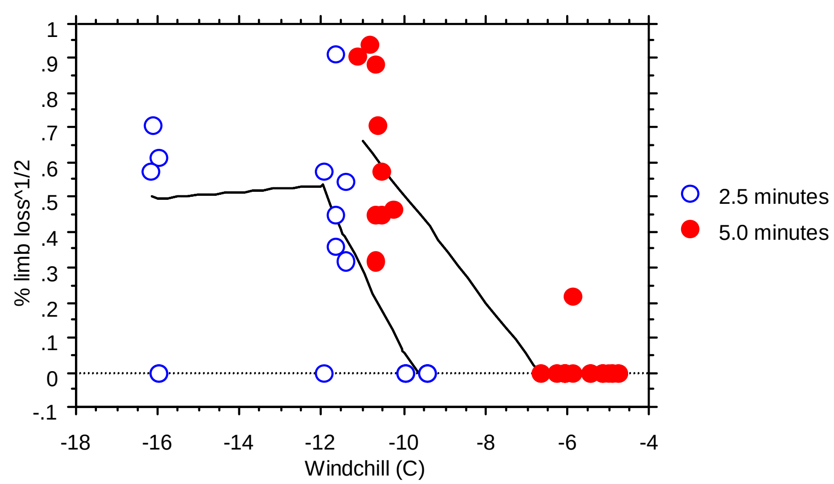


Table 3: Tukey/Kramer for %limb loss
Effect: treatment
Significance Level: 5 %
Inclusion criteria: Limb loss of surviving crabs, 5 minute exposure

S=Significant

	Mean Diff.	Crit. Diff.	
-2x16m/s, -2x8m/s	.023	.143	
-2x16m/s, -5x16m/s	-.517	.247	S
-2x16m/s, -6x8m/s	-.295	.165	S
-2x16m/s, control	.014	.143	
-2x8m/s, -5x16m/s	-.540	.247	S
-2x8m/s, -6x8m/s	-.318	.165	S
-2x8m/s, control	-.009	.143	
-5x16m/s, -6x8m/s	.222	.261	
-5x16m/s, control	.531	.247	S
-6x8m/s, control	.309	.165	S

Righting Response

Each crab's righting response was measured before and after treatment; each crab served as its own control with the change in the righting response the dependent variable. Only crabs that survived treatments were included in the analysis of righting response. The crabs that died following treatment were removed from the analysis. The righting response times followed a modified Pareto distribution, that is, a skewed distribution of positive values with a long right-hand tail. A Pareto distribution is a typical distribution for response times (B. Fagen, Univ. of Alaska Fairbanks, pers.comm). However, the righting response times were not measured past 300 seconds (assumption of no response past 5 minutes of no activity), which resulted in a bimodal Pareto distribution. The assumption of normality could not be met. Visual inspection of the distribution of residuals also disproved the assumption of homoscedascity. The lack of homoscedascity is mainly a function of the response times that are truncated at 5 minutes. Therefore, the severe treatments where all the crabs did not right themselves after 5 minutes had lower variances than the control and the less severe treatments. Non-parametric analysis of the change in righting response was necessary. In this case, a nonparametric analysis of variance, the Kruskal-Wallis test was used to test the hypothesis. The change in response times due to treatment was significantly different (p=0.002)(Table 5). A significant difference between the control and the least windchill treatment was not found with Dunnett's test (used for specifically comparing controls to other treatments), but a significant difference did exist between the control and the other three treatments (Table 5). A nonparametric multi-sample test, the Student-Newman-Keuls test, was performed to examine differences between specific treatments. Again, the control was significantly different from all but the least severe windchill treatment (Table 6). An overlapping similarity does not allow conclusions about treatment –2°C x 8m/s to be made. The remaining treatments are significantly different from the control but not significantly different from each other (Table 6).

Table 4. Kruskal-Wallis Test for change in righting response
Grouping Variable: treatment
Inclusion criteria: Surviving crabs with initial response

DF	4
# Groups	5
# Ties	0
H	22.145
P-Value	.0002
H corrected for ties	22.145
Tied P-Value	.0002

	Count	Sum Ranks	Mean Rank
-2x16m/s	12	333.000	27.750
-2x8m/s	11	203.000	18.455
-5x16m/s	2	74.000	37.000
-6x8m/s	7	263.000	37.571
control	13	162.000	12.462

Table 5: Dunnett test for control vs treatment righting response
Grouping variable: treatment Signifiance level: 5%
Inclusion criteria: Surviving crabs with initial response times

	Mean Diff.	Crit. Diff.	
-2x16m/s	184.126	107.775	S
-2x8m/s	37.212	110.293	
-5x16m/s	291.085	204.488	S
-6x8m/s	279.313	126.213	S

S=Significant

treatment

Table 6: Student-Newman-Keuls for righting response
Significance level: 5%
Inclusion criteria: surviving crabs with initial response times

	Mean Diff.	Crit. Diff.	
-2x16m/s, -2x8m/s	146.914	128.183	S
-2x16m/s, -5x16m/s	-106.958	154.178	
-2x16m/s, -6x8m/s	-95.187	128.183	
-2x16m/s, control	184.126	154.178	S
-2x8m/s, -5x16m/s	-253.873	169.865	S
-2x8m/s, -6x8m/s	-242.101	154.178	S
-2x8m/s, control	37.212	128.183	
-5x16m/s, -6x8m/s	11.771	128.183	
-5x16m/s, control	291.085	181.070	S
-6x8m/s, control	279.313	169.865	S

Because the cell counts are not equal, e harmonic mean is used to estimate n.

Evaluation

All experimental objectives to examine the effect of windchill on mortality, limb loss, and activity were met.

Mortality

Mortality increased with increasing severity of treatment. A threshold existed at a windchill exposure of –10.5°C; no mortality occurred at less severe exposures. Critical cell damage that results in death might occur even when a snow crab is exposed for 2.5 minutes at a windchill of –10.5°C. The rapid cooling associated with windchill may cause some failure at the cellular level. Ice crystals forming in the cytoplasm will disrupt cell membranes and cell death will occur. As heat loss approaches lethal limits, neurons are the first cells to fail (Prosser 1991). Critical neuron failure is a probable cause of death.

Two treatments (-5°C x 16 m/s and –6°C x 8 m/s) with similar calculated windchill values had significantly different mortality rates. The calculated windchill values for the two treatments were –10.5°C and –11°C. It is unlikely that that a physiological threshold operates in the range of half a degree of windchill. The problem may lie with the windchill calculation. The windchill measurements should be interpreted as an approximation of the cooling rate a crab would be experiencing. An exact calculation of cooling rate (and hence, windchill) is difficult because the area of the surface being cooled is necessary to construct constants. Snow crabs have spines and angular extremities with surface areas that are difficult to measure. Disagreement exists as to what 'windchill' actually measures and new formulae are being calculated to result in more precise estimates of cooling rates. The threshold between the two treatments of –5°C x 16 m/s and –6°C x 8m/s may be due to the windspeed. The higher windspeed in the former treatment may increase the rate of water loss and evaporation from the gill chambers thereby increasing the potential for freezing damage to the gills.

Mortality rates decreased when the exposure time was decreased. The reduced exposure time results in lower heat loss, less body cooling, and presumably less cell death and damage. The retention of seawater in the gill chambers may also be a factor. Insulating seawater may lessen freezing damage to the gills. Some other physiological response such as impaired oxygen delivery may cause death from rapid cooling, but the low demand for oxygen at cold temperatures among heterotherms and the quickness of the onset of mortality argues for neuronal cell damage.

Autotomy

Limb loss occurred at moderate to severe windchill, but was highly variable when it did occur. No significant difference occurred in amount of autotomy between the moderate and severe windchill treatments. Severe windchill treatments included death as a final response of the crabs to the treatments. Treatments that stressed a portion of the crabs to death complicated the results. Autotomy is a physiological response that could be compromised by severe windchill. Since other behaviors such as the righting response can be compromised, it is likely autotomy is compromised as well.

Why does autotomy, a predator escape response, result from a non-specific stressor like windchill? Autotomy of a limb initiates at the cleavage plane between the coxa and merus (Skinner 1985). Autotomy resulting from windchill exposure differs from natural, predator-induced autotomy. Windchill exposure could freeze or weaken multiple cleavage planes resulting in multiple limb loss. However, a more likely explanation is that the severe stress of rapid cooling generated an impulse which innervated the muscles involved with autotomy. A sharp blow to some crab species will initiate autotomy. Windchill-induced limb loss is more severe as multiple limbs can be lost following exposure. Windchill exposure is a non-specific stressor whereas predator-induced autotomy is directed. Multiple limb loss in populations of the congener *C. bairdi* is rare (Juanes and Smith 1995). *Chionoecetes opilio* may not have evolved to cope with multiple limb loss.

Limb loss does not necessarily preclude survival. A crab can survive over a year with no limbs (S. Rice, NMFS, pers. comm.). However, some level of limb loss may affect survival. Snow crabs have ten periopods: four walking legs and a cheliped on each side. The chelipeds are presumably the most important for survival with their multiple functions in feeding, defense, and mating (grasping of females). Our experiments suggest that the chelipeds are rarely lost as a result of windchill. The rear (4^{th}) walking leg was the most readily autotomized limb in our experiments, followed by any other walking leg. The loss of one walking leg would have a minimal effect on crab fitness. However, the additive effects of many lost limbs and the pattern by which they are lost will affect crab fitness. Multiple limb loss occurred in the experiments, and up to 80% were lost due to low windchill in some crabs. A snow crab with few limbs would likely have a low survival rate and low productivity.

Mating behaviors can be compromised by a reduced number of limbs (Smith 1992, St. Marie, pers. comm.). Behaviors observed in *C. bairdi* such as the ability to stand over a female, standing “high-on-legs”, kicking, and body lifting of the female above the male (Donaldson and Adams 1989) rely on leverage and balance, and may be difficult without a full complement of limbs. These behaviors are also common during antagonistic interactions with other males (Donaldson and Adams 1989). Limb loss could affect both mating behavior and competitive ability of male *C. opilio*.

Snow crabs have a behavior observed in the lab that may stem from predator avoidance that one of us (T. Shirley) also observed in red king crabs (and used to advantage in measuring their weights, as the crabs remain still for a moment while they are extended). Snow crabs similarly flare out their limbs when grasped. A fish predator that attempted to swallow the crab in the same fashion would be presented with a much larger effective prey size. The predator might have difficulty fitting the crab into its mouth with its limbs splayed out. A crab with no limbs or few limbs would be easier to consume than one with limbs (Smith 1995).

A relationship between the limited field data and the experimental data is difficult to assess. Examination of bycatch of 1718 *C. bairdi* during the 1999 snow crab fishery recorded a limb autotomy rate of 0.3% (NPMC 2000). This rate is well below that observed for moderate to severe windchill values observed in the lab (Shirley 1998). However, the 0.3 % rate was recorded just prior to release and should be considered the instantaneous rate of limb loss (or the rate of loss over the 3-4 minutes the crab was held).

Injury rates were assessed from data collected aboard catcher-processors during the 1997/1998 Bering Sea snow crab fishery. Over 14,000 non-retained snow crabs from 394 sampled pots were assessed for injuries (Tracy and Byersdorfer 2000). Injury rates varied among vessels from 7% to 44% of crabs sampled with an average of 24% (Tracy and Byersdorfer 2000). Autotomized legs were the most prevalent injury and comprised 59% of the total injuries. Major damage (cracked carapace, bent/torn limbs, chela damage) comprised

10.9% of the total injuries (Tracy and Byersdorfer 2000). The weather conditions were not recorded during the injury assessment samples (Tracy and Byersdorfer 2000). A few observers serving on commercial snow crab vessels have informally reported large numbers of limb loss, and referred to windchill exposed crabs as “frisbees”.

Injury rates reported from observer data should be considered the rate of instantaneous limb loss that occurs over the few minutes the crabs are held. Instantaneous limb loss in the laboratory study did occur, but snow crabs also autotomized limbs over 7 days of observation. Estimates of bycatch limb loss in the field are conservative.

Annual pot surveys by observers on fishing vessels assess the crab condition (and hence previous injuries). The rate of limb loss in discarded crabs should be evident in pot samples, as these crabs are captured again the following year. However, if injury and limb loss reduce survival, then estimates of bycatch limb loss assessed from initial damage to crabs will be conservative. The snow crabs in this experiment were handled very carefully after exposure to windchill treatments. Bycatch snow crabs are not handled as gently on a fishing vessel. Any rough handling would exacerbate limb loss.

Righting response

The righting response was impaired by all but the least severe windchill treatments. A threshold exists beyond a severity of exposure of –2°C and 16 m/s (~-6°C windchill) where the righting response is impaired. The nervous system is complex and slight damage can result in a loss of function. Shirley (1998, 1999) noted loss of coordination evident by increased righting response times after red king crabs and Tanner crabs were exposed to wind and cold. The righting response is a complex reflex requiring muscle coordination and balance and can be a sensitive measure of well-being of organisms (Shirley and Stickle 1982). Snow crabs exposed to windchill below –6°C have an impaired righting response. A snow crab with no righting response, in a state of chill coma, could be considered functionally dead.

The rapid cooling associated with windchill causes some failure at the cellular level; neurons are the first kinds of cells to fail (Prosser 1991). Critical neuron failure explains the functional death, or chill coma experienced by Tanner crabs and red king crabs at lower windchill exposures (Shirley 1998, 1999). Some red king and Tanner crabs in a state of chill coma recovered seven days post exposure (Shirley 1999). Several snow crabs with no righting response immediately after treatment in the less severe treatments recovered after seven days post exposure, suggesting the potential to recover from mild exposures. Snow crabs that survived severe to moderate windchill exposure were less likely to recover.

Loss of mobility was evident after windchill exposure. Little active movement was observed in crabs that survived windchill of less than –10°C. Only 4 of 8 surviving crabs from the –6°C and 8 m/s treatment were observed actively moving. Sporadic motion of the limbs with no net movement is an apt description of the remaining survivors. All crabs in the less severe windchill treatments could move actively even when no righting response was evident. Crabs that could move did so with jerky, uncoordinated motion of the walking legs. High “standing-on-legs” type travel was not noted. Crab movement could be described as lifting the walking legs, pushing and sliding the body across the substrate.

Impaired mobility could alter foraging efficiency, seasonal migration, and daily movement behaviors. However, little is known about movement patterns of C. opilio in the Bering Sea. A tagging and recapture experiment with C. opilio in the Gulf of St. Lawrence found that snow crabs could move up to 3 km in 48 hr (Brêthes et al. 1985).

Loss of mobility and coordination could make crabs more vulnerable to predation (Smith 1995). Cod (Gadus morhua) have been found to prey seasonally on snow crab in the northwest Atlantic and consume soft shell males of up to 110 mm carapace width (Robichaud et al. 1991). However, cod stomachs contained four times more toad crab Hyas spp. than snow crab, even when snow crab densities were much higher (Robichaud et al. 1991). While cod use visual and olfactory clues to hunt, there is evidence that cod are not capable of detecting buried prey (Brawn 1969). The difference in predation rates may be explained by behavior. Snow crabs bury in sediment for protection, while toad crab rely more on camouflage (Arnold 1968). Moreover, stomach samples from skates (which can detect buried prey) collected in the same area contained five times more snow crab than stomach samples of cod (Robichaud et al. 1991). If similar predator/prey relationships occur in the Bering Sea, high predation of discarded snow crab might be expected if discarding occurred during weather severe enough to impair the righting response. Heavy predation on *C. bairdi* and *C. opilio* occurred in the southeastern Bering Sea by the Alaska and Bering skates, wattled eelpout, Pacific cod, and four sculpin species (Jewett 1982).

Conclusions

Impact on snow crab stock

Mortality of snow crab bycatch from the directed crab pot fishery had been estimated at 24%; this estimate was incorporated into the rebuilding plan (NPMC 2000). Mortality rates of discarded snow crab in the field are dependent on the weather conditions at capture. No database of weather conditions exists with which to “groundtruth” the estimated bycatch mortality, the reported deadloss, and the laboratory windchill data. Weather data from coast guard buoy #4065, located 250 miles south of the fishing grounds, is the most reliable source of data. Average temperature, windspeed, and windchill can be calculated over the fishing season. However, fishing effort is not constant over all days of the season. Fishing effort (measured as the number of pot pulls) increased towards the end of the season (Moore et al. 2000). The snow crab fleet does not deliver crab on the same days, therefore, some fishing days will have more effort when more of the fleet is fishing. Weather conditions on those particular days will impact more crabs than others. If weather conditions are very severe, fishing may be postponed or halted. These factors complicate accurate estimates of bycatch mortality. Nevertheless, an estimate of bycatch mortality using these parameters could be possible with more data collection. The upcoming season could be an opportunity for on-board observers to collect data on sorting practices and equipment, and on-deck weather conditions.

Products

1. A summary of the research results was presented at the Crab 2001 Wakefield Symposium, held in Anchorage, Alaska January 17-20, 2001.

2. An oral presentation of tentative results was made at the Alaska Chapter, American Fisheries Society annual meeting in Fairbanks, Alaska on November 16, 2000.

3. A manuscript describing the results of the research was submitted to a peer-reviewed scientific journal in spring 2001.

4. Other upcoming symposia may provide a relevant forum to present this research.

Key Words

Snow crabs, *Chionoecetes opilio*, wind chill, mortality, autotomy, righting response,

PROJECT 4B: MORTALITY AND GROWTH INCREMENTS FOR SNOW CRABS

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Executive Summary

Project 4B was identified after the FY00 application as an important project for funding when unexpended funds became available during the two year grant period shortly after the collapse of the Bering Sea snow crab fishery. This project investigated differences in mortality, molting rates, and molt increments of new shell and old shell male snow crabs (*Chionoecetes opilio*) held in laboratory conditions for approximately one year. Old shell crabs were at least one year or more beyond terminal molt, while new shell crabs were assumed to be in the first year after the molt to maturity. No molting occurred during the study period by either crab group, so growth or molt increments could not be determined. Mortality of both groups of crabs was relatively constant throughout the experiment. Mortality, when expressed as percentage of mortality per day, was significantly greater for old shell crabs ($0.458 \pm 0.006\% \cdot day^{-1}$, mean ± one standard error) than that of new shell crabs ($0.126 \pm 0.002\% \cdot day^{-1}$) averaged over the year, when all mortalities related to “artificial” stresses such as temperature increases or low water flow rates were removed. Our laboratory estimates of mortality are not proposed to be valid proxies for natural field mortality, however the difference in mortality between new shell and old shell crabs is instructive and suggests the potential for high mortality during stressful years.

Purpose of Project

One of the impediments to completing a scientific basis for management of snow crabs is lack of basic life history information. The relative isolation of snow crabs from ports and laboratories in the North Pacific has impeded studies of the species. Most life history information on North Pacific stocks has been inferred from shipboard measurements made during the annual summer trawl survey. Although studies have been conducted on snow crabs in the eastern North Atlantic, these data should be applied with caution to North Pacific populations.

One life history attribute that is critical to most management strategies is knowledge of natural mortality of different life history stages. The amount of natural mortality incurred by crabs, particularly the old shell and very old shell crabs (those age groups perhaps more vulnerable to mortality), is essential to understanding the dynamics of the stocks, forecasting standing stock, and establishing rebuilding plans. Mortality values currently being used are only conservative estimates.

This project investigated differences in mortality, molting rates, and molt increments of new shell, and old shell male snow crabs (*Chionoecetes opilio*) held in laboratory conditions for one year. Old shell crabs are at least one year beyond terminal molt, while new shell crabs are presumably younger. Insufficient numbers of very old shell crabs (two or more years beyond the molt to maturity) were collected to treat them as a separate experimental group; no distinction was made between old shell and very old shell carapace condition. No “graveyard” condition (>36 months post ecdysis) crabs were collected.

Approach

Male snow crab of both shell classifications were collected alive in the eastern Bering Sea between March 22 and March 26, 2000, returned to the laboratory in Juneau, and were cultured in flow-through seawater tanks for approximately one year (May 30, 2000 until April 3, 2001). Crabs were held in tanks for eight weeks (March 27 - May 29, 2000) prior to assignment of individuals to experimental treatments to eliminate stress-induced mortality. Culture of crabs required daily inspection for damage, limb loss and mortality, and also for tank cleaning and monitoring of flow rates, temperature and salinity of seawater within tanks.

Crabs were screened for diseases, including hemolymph diseases, and individually numbered with plastic tags attached to the third or fourth walking legs with plastic cable ties. Carapace measurements and number of missing appendages were measured initially for all crabs. Supplemental measurements were made at the termination of the experiment. Crabs were fed on nonconsecutive days twice weekly with a mixed diet of squid, herring and cod, and were fed to excess. Sea water provided to crab tanks was from -30 m and followed ambient temperatures; temperature in the tanks ranged from 4.7°C to 8.8°C over the course of the experiment.

Crabs were monitored daily for limb loss, molting and mortality. Mortality of crabs was determined by observation of activity and movements of limbs particularly gill bailers (scathognathites). Dead crabs or lost limbs were removed and frozen for later analysis or disposal.

The study initiated with 52 old shell (OS) and 116 new shell (NS) crabs. No significant difference existed in the mean carapace width (CW) of the two groups at the beginning (NS = 99.9 ± 2.5 ; OS = 98.9 ± 3.2) or end (NS = 106.1 ± 3.1 ; OS = 104.8 ± 2.0) of the experiment. During the experimental period, 5 old shell and 31 new shell crabs suffered “non-natural” mortality resulting from equipment failures (interruption of sea water flow, caused by power outages and failure of the sea water system) and were removed from the total; these totals include all mortalities occurring within two weeks following the artificial stress. “Natural” mortality rates were observed for the remaining 47 old shell and 85 new shell crabs. A percentage of crabs that died from each group was recorded daily from day 0 to termination of the experiment on day 309. ANOVA was used to test for significant differences in mortality between the groups.

Results, Evaluation and Conclusions

No crabs molted during the course of the study, so growth increments could not be determined. Also, no tests for differences in molting or growth increments could be made between old shell and new shell crabs. Lack of molting by the snow crabs was not unusual, particularly for a study spanning a single year. Anecdysial molting by *Chionoecetes* spp. has rarely been substantiated by large scale, in situ tagging studies. However, molting by mature *Chionoecetes bairdi* after 24-28 months of laboratory culture was reported (Paul and Paul 1995). Similarly, decadal changes in the probability of molting for *Chionoecetes bairdi*, based on interannual variations in size-frequency distributions of crabs collected in the annual NMFS survey, was reported (Zheng et al. 1998). Less information exists on molting probabilities of *C. opilio*.

Mortality of both groups of crabs was relatively constant throughout the experiment (Figure 1). Mortality, when expressed as percentage of mortality per day, was significantly greater for old shell crabs ($0.458 \pm 0.006\% \cdot day^{-1}$, mean ± one standard error) than that of new shell crabs ($0.126 \pm 0.002\% \cdot day^{-1}$) throughout the year. We removed from consideration all mortality related to "artificial" stress such as temperature rises or low water flow rates.

In a related study, significant differences in feeding rates occurred between the groups; new shell crabs consumed more than old shell crabs (Shirley and Warrenchuk, project 4B in this report). One interpretation is that new shell crabs were more fit; i.e., they had higher energy stores and were less susceptible to stress-induced mortality.

Long term culture of marine organisms is notoriously difficult; maintenance of deep water crabs in laboratory conditions for almost a year should not be expected to produce results approximating those occurring in response to natural conditions. Laboratory environmental conditions, including water temperature, salinity, dissolved oxygen, hydrostatic pressure, light intensity and quality, quantity and quality of diet, competitive interactions and predation, might all be expected to vary considerably from what the crabs would experience in situ. However, often laboratory experiments provide one or the only viable method of approximating a life history variable. We feel that our measurements of crab mortality measured in the laboratory should not be considered as proxies for natural mortality. However, we do expect that the differences in mortality between our experimental groups (at some level) depict differences that exist in nature; i.e., old shell snow crabs have higher mortality rates than new shell snow crabs and have decreased capability to respond to stress.

Products

An abstract containing this research was accepted at an international crab meeting, Life Histories, Assessment and Management of Crustacean Fisheries, to be held in Coruna, Galicia, Spain, October 8-12, 2001, however the presentation will not be made because of travel hazards created by recent terrorist activities. This research may be published in the proceedings of the meeting, in the journal Fisheries Research; if not, it will be published in a peer-reviewed scientific journal. Results of the research will be presented at the Interagency Crab Meeting, to be held in Anchorage, Alaska, December 12-14, 2001.

Key Words

Snow crabs, *Chionoecetes opilio*, mortality, molting, growth

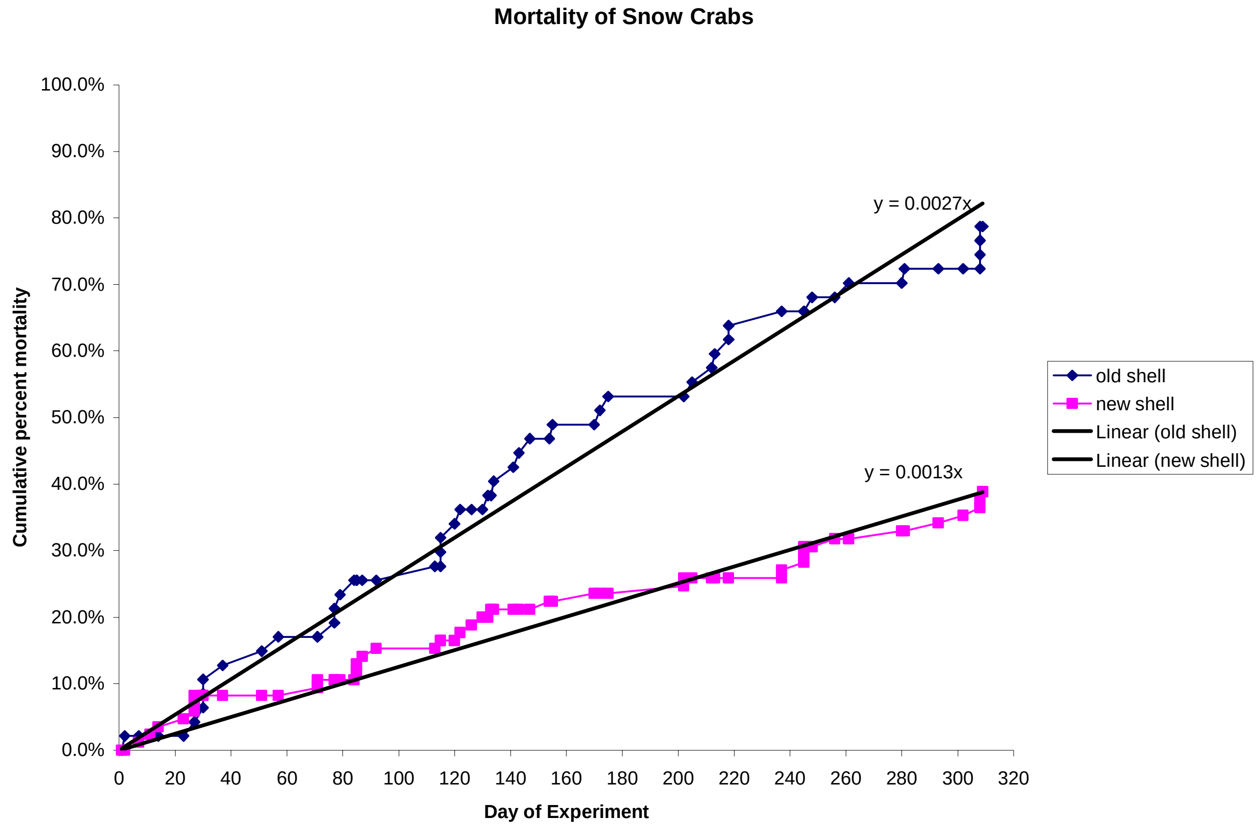

Figure 1. Cumulative mortality of new shell and old shell male snow crabs in laboratory conditions from May 30, 2000 through April 3, 2001.

PROJECT 4C: FEEDING AND ENERGETICS OF SNOW CRABS

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Executive Summary

This laboratory experiment compared feeding rates and energetics of old shell and new shell snow crabs (*Chionoecetes opilio*) to examine possible differences in their mortality rates and their ability to respond to environmental variability. Comparisons were made of energy intake (feeding rates and absorption efficiency) and energy allocations to different organ systems of newly mature and older male snow crabs. The amount of energy allocated to growth, reproductive products or reserves was compared for the different life history stages.

Purpose of Project

The aim of this experiment is to test for differences in food intake and absorption efficiency and to relate these energetic measurements to changes in weight, activity, growth and mortality rates of snow crabs over the course of a year.

Alaskan snow crabs (*Chionoecetes opilio*) in the Bering Sea have supported one of the world's largest and most valuable crab fisheries in recent decades. Harvests commonly exceeded 200 to 300 million pounds annually, until a drastic decrease in biomass of mature crabs resulted in sharply lower harvest quotas beginning in 2000. Declines in snow crab harvests have had serious economic and social implications for Alaska.

Under the mandates of the Magnusun-Stevens Fisheries Conservation Act (MSFCA), the decline of mature biomass of Bering Sea snow crabs to below 50% of maximum sustainable yield (MSY) resulted in their being designated as "overfished" by the North Pacific Fisheries Management Council. Development and implementation of a Fisheries Rebuilding Plan required by MSFCA, demonstrated that one of the impediments to a scientific basis for management of snow crabs was the lack of basic life history information. An understanding of the mechanisms resulting in the recent declines cannot be achieved without additional information on the biology of the species.

Few laboratory or field experiments have been conducted with Alaskan snow crabs, probably because their collection for studies has been impeded by their relative isolation from ports and laboratories in the Eastern Bering Sea. Most life history information on North Pacific stocks has been inferred from shipboard measurements made during two relatively narrow temporal windows, either during the annual NMFS summer trawl survey, or from data collected by observers during the progress of the commercial fisheries in winter. Although laboratory studies have been conducted on snow crabs from the eastern North Atlantic, these data should be applied with caution to North Pacific populations because of potential differences in genetic traits and differing habitat and hydrographic conditions.

Snow and Tanner crabs belong to the family Majidae, whose male and female members enter anecdysis with their molt to maturity, or cease molting. The molt to maturity may occur over a range of sizes and is accompanied by an increase in the relative size of the major chela for males, which confers advantage during mate competition. A smaller, mature male has competitive advantage over larger, immature males. Larger, mature males also have competitive advantage over smaller, mature males, so that delaying the molt to maturity until a larger size is attained may confer selective advantage if life expectancy is high, but might be selected against during times of low survival. The variation in crab sizes at the time of molt to maturity and the accompanying anecdysis results in a larger number of size and shell conditions in mature males than might be found in other species.

Post anecdysial molting has been documented to occur in laboratory conditions (Paul and Paul 1995), and has been inferred to have occurred in the field from analysis of annual size-frequency distributions (Zheng et al. 1998). For practical considerations the probability of additional molting after the molt to maturity is extremely low (for a comprehensive review, see Conan and Comeau 1986, Conan et al. 1988, Donaldson and Johnson 1988). With increasing time since the terminal molt, the carapace of the crab becomes progressively fouled. Because no practical technique exists to accurately age crabs, they are categorized as new shell, old shell, and very old shell, depending upon the condition of their carapace and the degree of fouling. A pervasive belief is that very old shell males and old shell males have decreased life expectancy; large numbers of old shell males in a population could result in the rapid collapse of a fishery. This experiment was part of a test of that hypothesis, with laboratory measurements to identify mechanisms in which energetics might affect the survival of old shell crabs.

One life history attribute that is critical to most management strategies is knowledge of natural mortality of different life history stages. The amount of natural mortality incurred by crabs, particularly the old shell and very old shell crabs (those age groups assumed to be more vulnerable to mortality), is essential to understanding the dynamics of the stocks, forecasting standing stock, and establishing rebuilding plans. Understanding the biological causes of the mortality is also critical. If the old shell crabs are less able to able to withstand stress (from any means, e.g., variations in water temperature, salinity, food supply or handling during the commercial fishery), their mortality might be more predictable. Could physiologically stressful years (e.g., changes in temperature, food or a combination) result in mass mortality of old shell males in some years but not in others? An alternate concern is that older crabs might have smaller energetic investments to gonads, with proportionately decreased mating capability or reproductive output.

Approach

In a related study, mortality of old shell and new shell snow crabs was measured for approximately one year (Shirley and Warrenchuk, Project 4B in this report). In this project, we measured growth (measured as changes in weight), activity, feeding rates, and absorption efficiency of old shell and new shell, male snow crabs. We also measured the energy content for specific organ systems within male snow crabs of different carapace conditions. Tissues analyzed for energy content included somatic muscle, digestive gland (hepatopancreas), the tissues primarily used for energy storage in decapod crustaceans, and gonads. The wet and dry weights of the remaining digestive system (foregut, midgut and hindgut), carapace, and gills were measured to assess differences between new and old shell crabs which might occur either in organ weights or in percentage contribution to total body mass.

Legal males (>101 mm CW) and smaller, mature males, of both new shell and old shell carapace conditions, were used in the experiments. Crabs were collected from a chartered commercial crabbing vessel that delivered the crabs to Dutch Harbor, Alaska. A research technician supervised the sorting and handling of crabs aboard ship as they were brought up in crab pots. Crabs were shipped by airfreight (excess baggage, to insure that they accompanied the technician on the return flight) alive from Dutch Harbor, Alaska, and transferred to seawater holding tanks immediately upon arrival in Juneau.

In the laboratory, male crabs were held in flow-through seawater tanks and fed ad libitum for eight weeks prior to their use in experiments to identify any that may have been weakened by shipment. The seawater intake for the laboratory is at –30 m in Auke Bay and is reasonably similar in salinity and temperature for much of the year to ambient waters which the crabs are exposed *in situ*, except that in some years the water temperature is two to four degrees warmer. As in previous studies, crabs were screened for bitter crab disease (BCD) by examination of serum samples, and any diseased crabs were excluded from the experiment. Screening for BCD was via microscopic examination of serum samples. Crabs were numbered by attached Floy tags to the merus of the fourth or fifth pereopod with a plastic cable tie. Carapace width and length, chelae height, carapace condition, number of missing or damaged appendages, and other injuries to crabs were recorded for each crab prior to the beginning of the experiment. Tanks were inspected daily for crab mortality and water flow. Water temperature was recorded daily in each tank by means of digital thermometers; water temperature was also recorded daily at the NMFS Auke Bay Labs dock. Temperature in the tanks ranged from 4.7°C to 8.8°C over the course of the experiment.

Tanks were covered with opaque lids to decrease disturbance, but large laboratory windows permitted the experiments to mimic seasonal changes in ambient photoperiod. No strict controls were placed on the laboratory photoperiod, however, little activity occurred during the evening or night; the laboratory was locked and without night activities.

One measure of well being of the crabs is an activity index. Activity of crabs was examined by measuring the righting responses of the crabs at the beginning of the experiment and monthly for the duration of culture. Crabs were placed on their dorsum and the time required for them to right themselves was measured in seconds to a maximum of 180 seconds. The righting response is a complex reflex requiring muscular coordination and balance and can be a sensitive measure of well being of organisms (Shirley and Stickle 1982). Previously I found significant relationships between righting response and windchill values with snow crabs, Tanner crabs and red king crabs. Each crab served as its own control; that is, changes in righting responses over the course of the year were assessed against initial righting responses. Crabs performing poorly in righting response were watched carefully for mortality or were eliminated from experiments.

Mortality was assessed by examination for movement of periopods, mandibles, maxillae and scaphognathites; mortality was recorded daily.

Crabs were fed a mixed diet of squid, herring or salmon, and mussels *ad libitum* twice weekly for a 24 hour period for the duration of the experiment to help insure a balanced diet. Crabs are notoriously irregular and messy feeders and consistent data are difficult to collect. We measured differences in wet weights of food placed in tanks and removed 24 hours later; each crab was fed within a separate compartment. Controls items of each type of food were placed in tanks for 24 hr and differences in their weight measured to account for water uptake and losses to dissolution.

Handling of the crabs was kept to a minimum, however the tanks had to be cleaned after each feeding, and also scrubbed and treated with disinfectants on a regular basis to curtail laboratory infections. During periods of tank disinfecting, crabs were transported in containers of water to adjacent, previously cleaned tanks.

We tested for differences in food intake and absorption efficiency and related these energetic measurements to changes in weight, activity, growth (changes in weight) and mortality rates of crabs over the course of a year. The experimental design included two groups of 10 male crabs each, with each replicate group positioned into one or more tanks randomly placed within the laboratory. A stratified, random sampling scheme was used to insure that similar sized crabs were included within each replicate.

This experiment measured the energy content of selected tissues and organ systems within the different carapace classes. Energy content (measured in calories per g dry weight) was measured by means of a semi-microbomb calorimeter. We have had excellent success measuring energy content of a variety of marine organisms in our lab, including krill, capelin, sandlance, salmon and pollock. Crabs were sacrificed and tissues excised, dry weights measured, and caloric values measured for at least 15 crabs within each carapace class. Crabs were sacrificed at the beginning of the experiment, and again at the termination of the experiment for all measurements.

Initially the project included sampling 10 male crabs of each shell condition; however, as part of an additional study within the current year we subsequently increased the sample sizes of specimens of male crabs being analyzed. Additional males of each shell condition (new shell and old shell) were collected at the beginning and termination of energetic studies and been kept frozen at -20ºC. These specimens were dissected and tissues analyzed for percent weight contribution and caloric content beginning in July, 2001. The results are included here for completeness.

At the termination of the experiment, seawater tanks were cleaned with antiseptic agents. Many of the crab carcasses were chemically digested, dried, baked or combusted. Remaining crab carcasses, currently frozen, will be properly disposed of at the ADF&G Tag Laboratory.

Results, Evaluation and Conclusion

All experimental objectives to examine differences in food intake and absorption efficiency for new and old shell crabs were met satisfactorily.

Significant differences in feeding rates occurred between the groups. New shell crabs consumed more than old shell crabs throughout the year (Fig. 1), whether consumption was expressed as grams of food/crab/day (Table 1) or grams of food/gram crab/day (Table 2). New shell crabs consumed significantly more food per day than old shell crabs. A significant difference existed between the amount of herring and cod consumed between crab types. The amount of squid consumed was not significantly different between new shell and old shell crabs. When consumption was adjusted for crab size, new shell crabs consumed more food per gram of crab weight (wet) than old shell crabs. New shell crabs consumed more herring and cod than old shell crabs, but no significant difference existed in the amount of squid. The feeding rates of both groups (all food types combined) plotted over time resulted in significant regressions (Fig. 1), but the relationship is an artifact of the experiment timing and seasonal changes in water temperature.

Table 1. Daily consumption of snow crab (grams of food per crab per day).

	New shell (g food/day)	Old shell (g food/day)	t-test (new vs old shell)
Herring	1.06 ± 0.14	0.51 ± 0.09	p=0.014, significant
Cod	2.88 ± 0.27	1.74 ± 0.16	p=0.005, significant
Squid	1.19 ± 0.12	1.02 ± 0.124	p=0.349, not significant
All food types	1.67 ± 0.12	1.06 ± 0.08	p<0.001, significant

Table 2. Daily consumption scaled to crab size (grams of food per day per gram of crab). The amount of food eaten was scaled to individual wet weight of crabs (not adjusted for lost limbs).

	New shell (mg food/day/g crab)	Old shell (mg food/day/g crab)	t-test (new vs old shell)
Herring	2.21 ± 0.26	1.19 ± 0.20	p=0.042, significant
Cod	6.17 ± 0.58	4.38 ± 0.44	p=0.016, significant
Squid	2.53 ± 0.25	2.54 ± 0.31	p=0.976, not significant
All food types	3.56 ± 0.26	2.63 ± 0.21	P=0.006, significant

Only a single previous publication has addressed the relationship between temperature and feeding. For snow crabs from the Canadian Atlantic coast, food consumption increased with increasing temperature to 6°C, but decreased at higher temperatures and ceased above 12°C (Foyle et al. 1989). In our experiments, crabs were fed twice weekly, as opposed to once weekly or every two weeks for lower temperatures by Foyle et al. (1989). In our experiments, maximal feeding occurred at the highest temperatures (approximately 8°C), and consumption declined as temperatures declined to 4°C. Alaskan snow crabs most likely are acclimated to warmer water temperatures than those of the North Atlantic and might be expected to have optimal physiological responses near their acclimation temperatures.

Feeding rates were initially high for both groups of crabs, but declined continuously throughout the year for both groups. Decreases in feeding rates probably were related to several phenomena. The crabs were not fed for the initial several weeks after capture, a common precaution to decrease stress while being introduced to culture; an increase in feeding rates would be expected in response to food deprivation. Also, food availability in culture may exceed food availability in situ, and the crabs may have fed to satiation to replenish natural deprivation. However, the long-term decrease in feeding was most likely related to decreases in water temperature. Culture tanks were maintained with ambient water from -30 m depth, and in situ temperatures declined with the onset of winter (Figure 1).

No significant difference existed in the mean carapace width (CW ± Standard Error, S.E.) of the two groups at the beginning (NS = 99.9 ± 2.5; OS = 98.9 ± 3.2) or end (NS = 106.1 ± 3.1; OS = 104.8 ± 2.0) of the experiment. Similarly, no significant differences existed in the mean wet weight (grams ± S.E.) of the two groups at the beginning (NS = 370.7 ± 26.5; OS = 387.4 ±

35.0) or end (NS = 519.2 ± 45.8; OS = 487.4± 27.2) of the experiment (Table 3). A more accurate method of examining weight changes in crabs is to compare dry weights, since wet weight can vary greatly with stage of molt cycle or changes in osmotic environment. Similarly, no significant differences existed in the mean dry weight (g ± S.E.) of the two groups at the beginning (NS = 103.6 ± 8.4; OS = 105.5 ± 10.4) or end (NS = 114.6 ± 7.4; OS = 107.4 ± 9.0) of the experiment (Table 4). Although the increases in both wet and dry weight from the beginning to the end of the experiment might be construed to represent gains in tissue weights, the crabs were from different groups and inferences concerning growth cannot be made. Differential mortality of smaller crabs over the course of the experiment would result in larger averages.

Table 3. Wet weights of snow crabs at the beginning and end of the experiment. Some crabs were missing limbs; both dry and wet weights reflect the estimated weights of whole crab. If a limb was missing, the opposing limb was weighed and added to the weight of crab. If pairs of limbs were missing, limb weight was estimated from similar-sized crabs and added to the weight of crab.

Stage	New shell wet wt. (g)	Old shell wet wt. (g)	t-test (new vs old shell)
Initial	370.7 ± 26.5	387.4 ± 35.0	p=0.411, not significant
End	487.4 ± 27.2	519.2 ± 45.8	p=0.279, not significant

Initial NS vs End NS: p=0.003, significant difference
Initial OS vs End OS: p=0.015, significant difference

Table 4. Dry weight of whole snow crabs. Missing limbs were estimated as in Table 1.

Stage	New shell dry wt. (g)	Old shell dry wt. (g)	t-test (new vs old shell)
Initial	103.6 ± 8.4	105.5 ± 10.4	p=0.443, not significant
End	114.6 ± 7.4	107.4 ± 9.0	p=0.287, not significant

Initial NS vs End NS: p=0.153, not significant
Initial OS vs End OS: p=0.448, not significant

Significant increases in mean hepatopancreas wet weight occurred for new shell crabs, but not for old shell crabs over the year (Figure 2). Both groups had increases in mean digestive tract wet weight and gill tissue weight over time, with no differences between the groups (Fig. 2). Both groups had decreases in wet weight of gonads between the beginning and end of the experiment, with new shell having lower gonad weights at the beginning and end of the culture period (Figure 2). The decreases in gonad weight may be related to seasonal variation in reproductive cycles. Seasonal cycles of gonad development are poorly known for most crab species, particularly for males. Male Dungeness crabs in southeastern Alaska had significant seasonal changes in weight of gonads when expressed as a gonadosomatic index (weight of gonad/weight of crab) to account for variation in crab sizes (Swiney and Shirley 2001). The decline in gonad weight for snow crabs may have been an expression of an annual cycle, or may have been a result of laboratory culture.

A more accurate depiction of tissue weights is obtained from comparison of tissue dry weights (Fig. 3). The trends are similar to that of wet weights, except that the increase in hepatopancreas dry weight is more pronounced for new shell crabs than was obvious for wet weights, and the dry weight of hepatopancreas decreases for old shell crabs. Gonad tissue and somatic tissue show little change except for a small increase in somatic tissue for new shell males.

Energy content (joules/mg) of hepatopancreas and somatic tissues did not vary between new shell and old shell crabs or over time (Fig. 4). The energy content of somatic tissue that we measured for snow crabs was similar to that of Tanner crabs (Paul and Fuji 1989). The energy content of gonads was higher for new shell crabs than old shell crabs at both the beginning and end of the study; however, both groups declined in energy content, similar to their decrease in gonad weight.

The wet weight of gonad tissue expressed as a percent of crab total wet weight (Fig. 5) was approximately an order of magnitude higher than reported for Dungeness crabs (Swiney and Shirley 2001).

Absorption efficiencies had large variances, perhaps related to the lack of consistency of food items (Table 5). The food items were not homogenized for ash determinations because they were not homogenized when presented as food to the crabs. No patterns were evident, except that old shell crabs had higher absorption efficiency than new shell crabs with squid. The absorption efficiencies for all food types of new shell and old shell crabs (21.0 and 22.6%, respectively) were not significantly different. Although the absorption efficiencies are low, they fall within reported ranges (Paul and Fuji 1989).

Table 5. Absorption efficiency determined by Conover Ash Ratio Technique (Conover 1966). Percent organics of Pacific herring (86.3% ± 0.1 %) and Pacific cod (83.9 % ± 0.3 %) are from Anthony et al. (2000); squid composition measured in our lab.

Food type	New shell absorption efficiency (% organic absorbed)	Old shell absorption efficiency (% organic absorbed)	t-test (new vs old shell)
Herring (1)	11.9 ± 10.9	5.3 ± 4.9	p=0.608, not significant
Cod (2)	39.3 ± 29.5	21.6 ± 9.2	p=0.539, not significant
Squid (3)	11.9 ± 2.1	41.2 ± 6.0	p=0.01, significant
All food types	21.0 ± 10.2	22.6 ± 6.1	p=0.897, not significant

Only a few prior studies have addressed energetics of snow crabs (Foyle et al., 1989; Sainte-Marie, personal communication). Most of the energy consumed by snow crabs (Foyle et al. 1989) and Tanner crabs (Paul and Fuji 1989) is used for maintenance (respiration), accounting for approximately 60% of total energy expenditure. Interestingly, the low temperature break-even point (where energetic intake equaled expenditures) occurred around 1^{o}C, even though crabs are commonly found in colder temperatures (Foyle et al. 1989). The break-even point might be higher for snow crabs in Alaska, where average water temperature is higher than on the Atlantic coast of Canada. If so, old shell crabs might be subjected to prolonged energy deficits during colder than average winters, and higher mortality might result.

This study substantiates that old shell, male snow crabs eat less and are more lethargic than new shell crabs. They also have higher mortality rates and are more susceptible to stress when exposed to changes in temperature or oxygen content. The higher mortality rates of old shell males appear to be related to a loss in energy reserves resulting from decreased feeding rates and perhaps absorption efficiency. The available consumed energy may have been allocated for gonadal development at the expense of maintenance of other organ systems or energy reserves. Physiologically stressful years (e.g., variations in temperature, food, oxygen content, or some combination) may result in mass mortality of old shell crabs, or result in old shell crabs having smaller contributions to reproduction. One interpretation of our results is that new shell crabs were more fit; i.e., they had higher energy stores and were less susceptible to stress-induced mortality.

Products

Two semiannual progress reports and a final report were generated as part of the reporting requirements for this research. Additionally, at least one manuscript will be prepared for submission to a peer-reviewed scientific journal, such as the Journal of Crustacean Biology, the Canadian Journal of Fisheries and Aquatic Sciences, or the U.S. Fishery Bulletin. Dissemination of project results will also be made via presentations at scientific meetings at state and international levels. An oral presentation will be made at the Annual Interagency Crab Research Meeting in Anchorage, scheduled for December 12-14, 2001. An additional presentation will be made at a future international meeting of a professional society meeting such The Crustacean Society.

Acknowledgments

We especially thank Lynnette McNutt for assistance in all phases of the project. Dr. Adam Moles, NMFS, is also thanked for his advice and logistical assistance. The NMFS Auke Bay Lab generously allowed us access to their facilities when the university seawater system failed in June, 2000; the project would not have been completed without their assistance. Thanks to Rance Morrison and his staff, Alaska Dept. of Fish and Game, Dutch Harbor; Larry Boyle and Ryan Burt from the Observer Training Center, and Gordon Blue and the crew of the F/V *Zolotoi* for support in crab collections.

Key Words

Snow crab physiology, feeding, growth, energetics

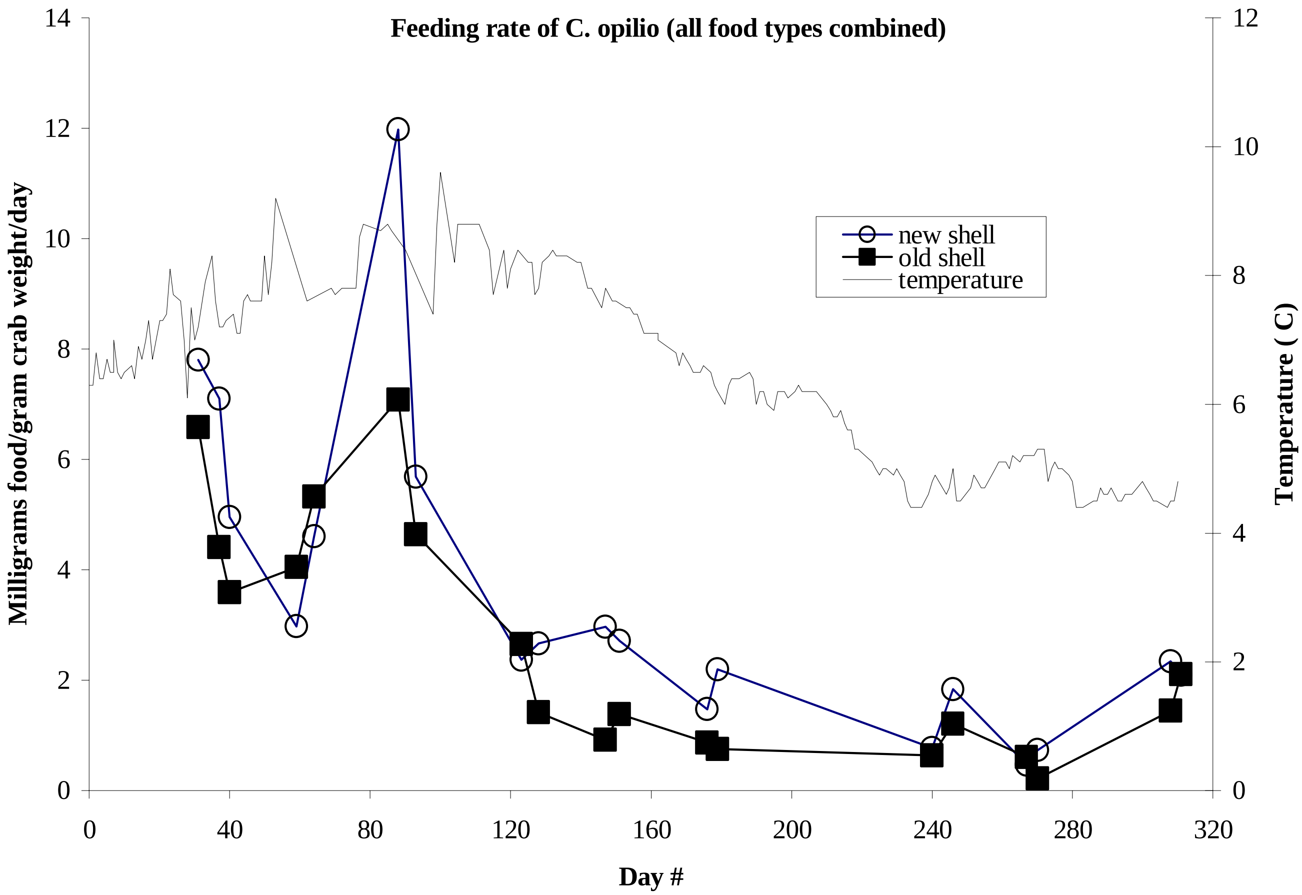

Figure 1. Feeding rates of new shell and old shell *C. opilio* expressed as milligrams of food eaten per gram of crab weight per day and daily tank water temperature over the course of the experiment.

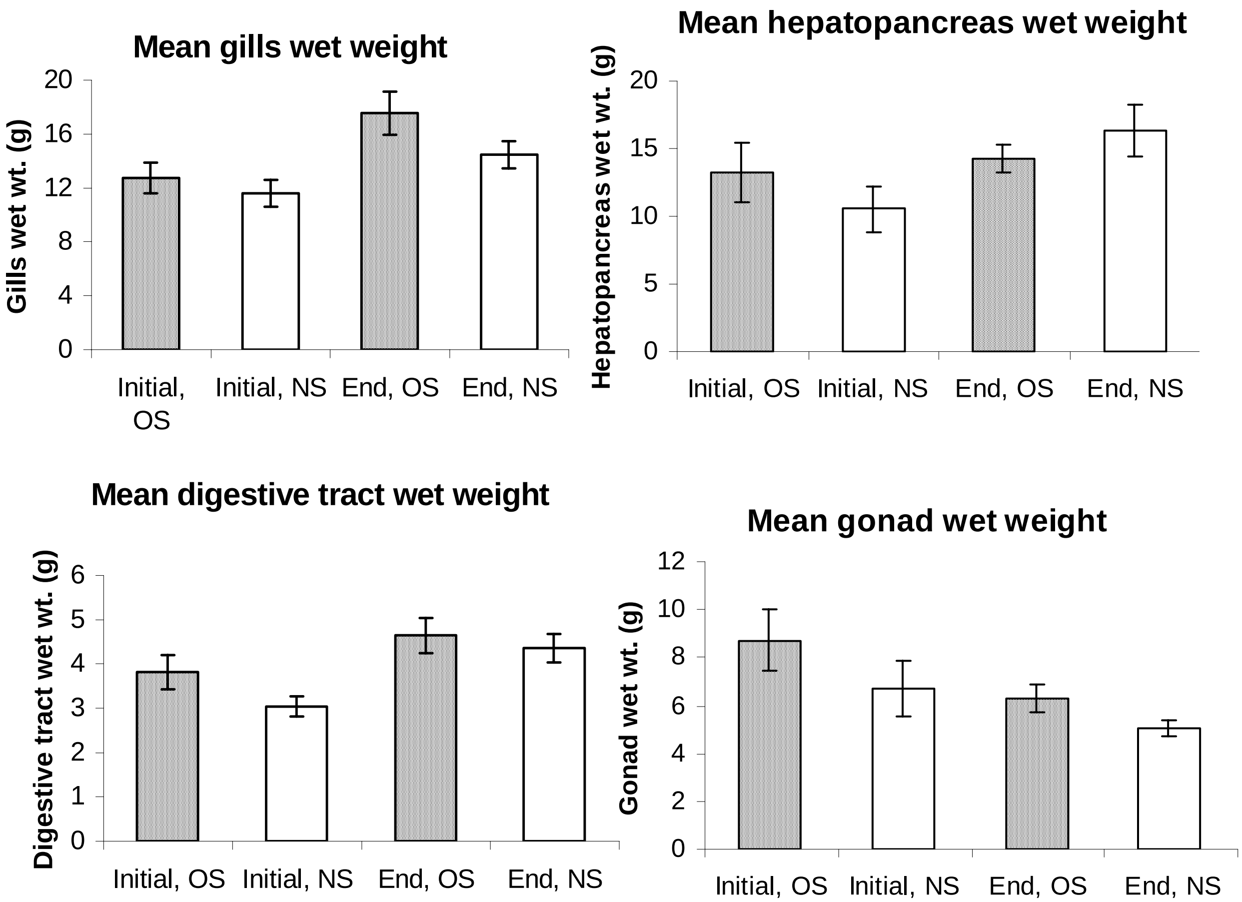

Figure 2: Comparison of mean wet weight of several organs and tissues of old shell and new shell *Chionoecetes opilio*.

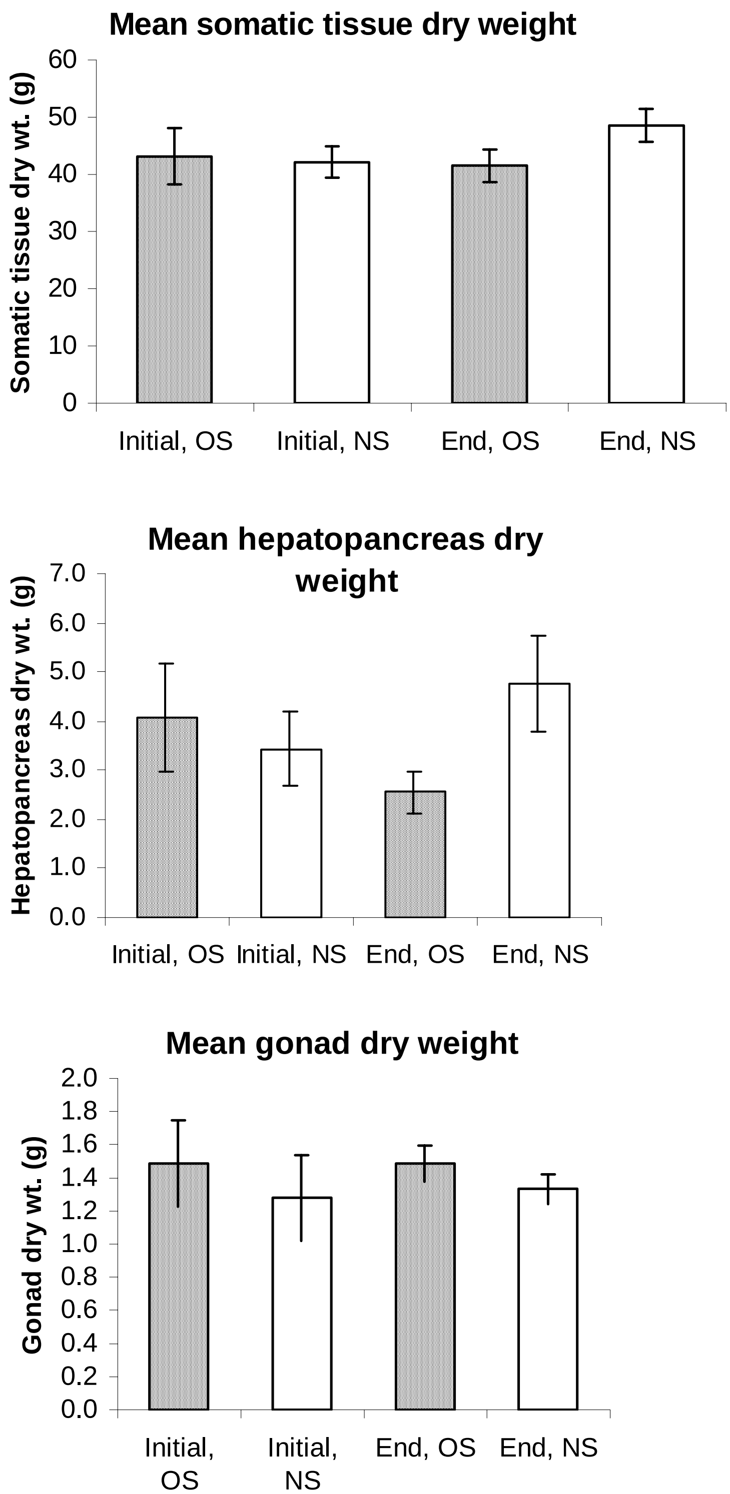

Figure 3: Comparison of mean dry weight for several tissues of old shell and new shell *Chionoecetes opilio*.

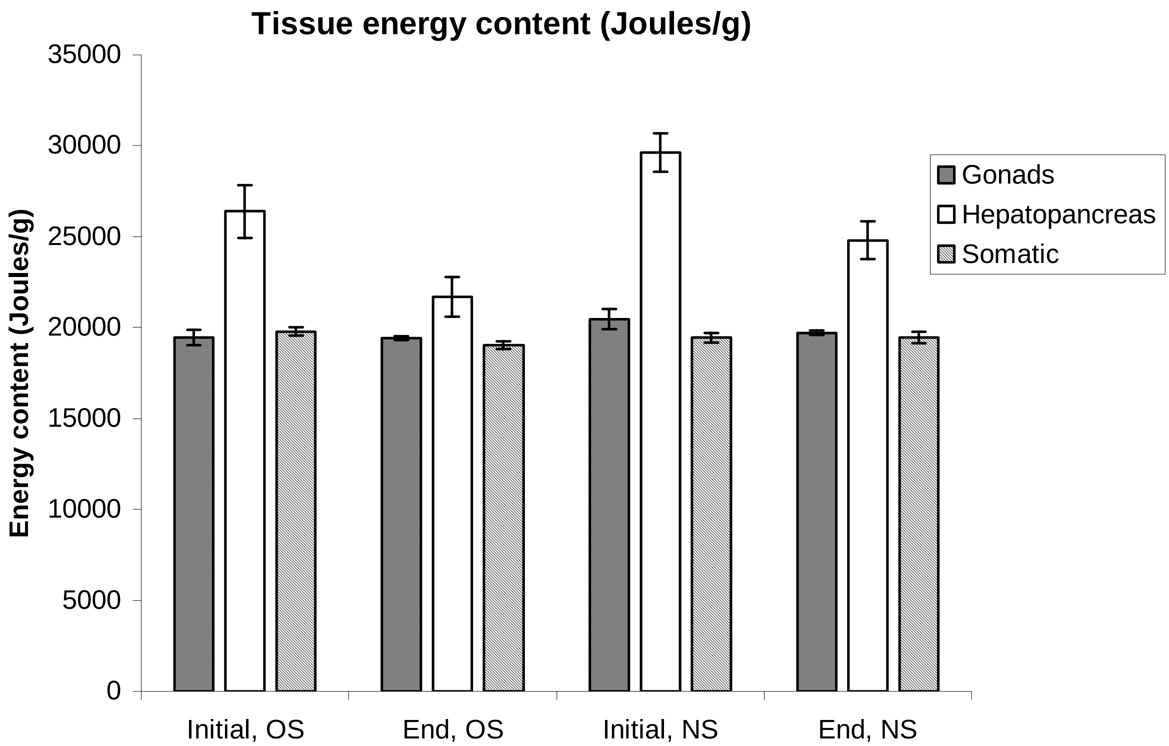

Figure 4: Comparison of energy content in tissues of old shell and new shell *Chionoecetes opilio*.

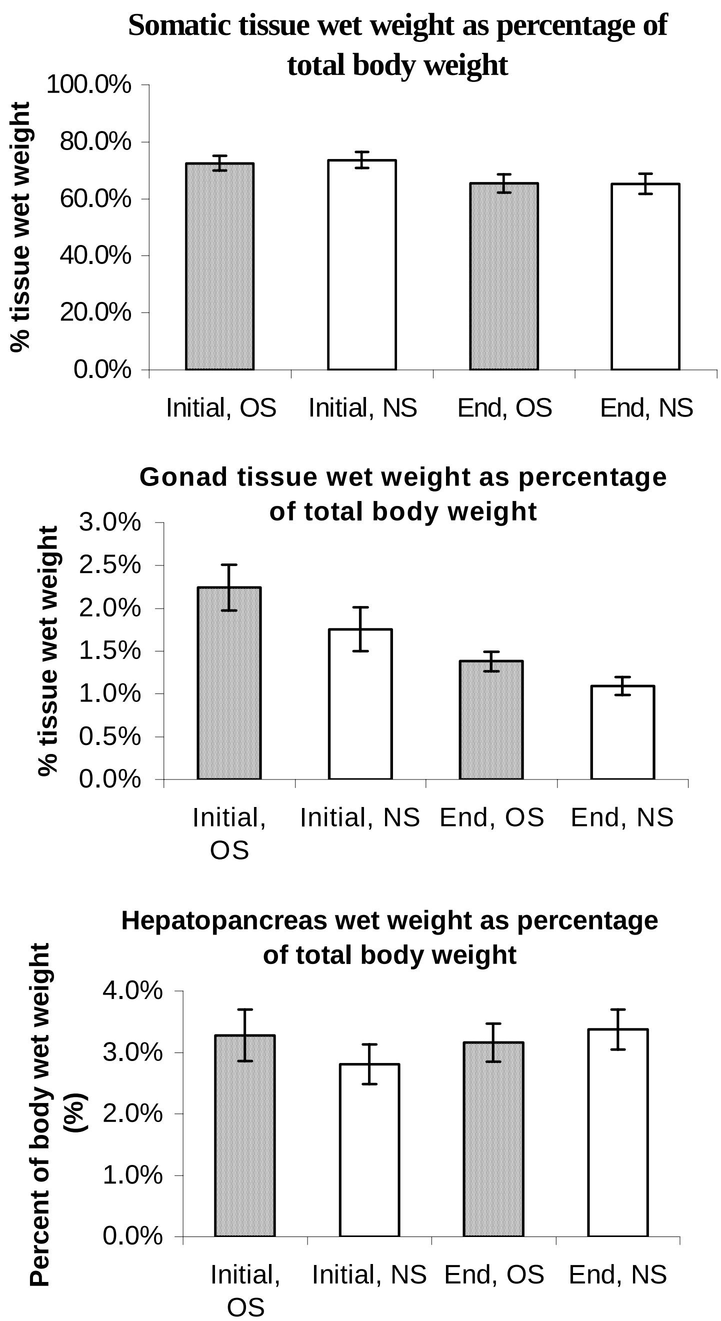

Figure 5. Mean wet weight of tissues expressed as a percentage of the total body wet weight of old shell and new shell *C. opilio*

PROJECT 5: EFFECTS OF TEMPERATURE ON TANNER CRAB GROWTH

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Executive Summary

This project examined the effect of temperature on intermolt durations of juvenile and adolescent male *Chionoecetes bairdi* Rathbun with carapace widths (CW) of 11-105 mm. Juveniles were identified by the absence of spermatophores and adolescents by their presence. The relationship between male maximum CW and intermolt duration exhibited a linear relationship for juveniles described by the equation: Intermolt period (degree days) = 7.9(CW mm) + 272; r^2 = 0.53. As males began to produce spermatophores at about 55 mm CW intermolt durations increased and the analogous equation was Intermolt period (degree days) = 7.6 (CW mm) + 1,215; r^2 = 0.14. A 55 mm CW spermatophore bearing male took about 2.5 times longer to molt than a similar sized juvenile.

This study also examined the effect of temperature on the intermolt duration of juvenile *C. bairdi*. The average length of the intermolt period for Gulf of Alaska male Tanner crabs, with carapace widths (CW) of 24-26 mm, was ascertained for captives held at 3°, 6° and 9°C. No test specimens died, and regardless of rearing temperature they fed avidly during captivity. Molting intervals were summarized in units of days and degree days. Increasing the rearing temperature from 3° to 6°C reduced the number of days between molts, but raising it to 9°C did not result in a similar reduction. The intermolt periods were 154 (SD = 24), 78 (SD = 14) and 74 (SD = 8) days for juveniles held at 3°, 6°, and 9°C, respectively. The number of days between molts for the 3°C test group was significantly longer than the intermolt duration of juveniles reared at 6° and 9°C. There was no significant difference in the number of days between molts for the 6° and 9°C groups. In units of degree days, the mean intermolt periods at 3° and 6°C were nearly identical 462 (SD = 74) and 467 (SD = 87), respectively. At 9°C molting occurred after an average of 665 (SD = 73) degree days. At 3° and 6°C degree-day intermolt durations were not significantly different, but at 9°C the intermolt duration was significantly longer than those observed at 3° and 6°C. Thus, at 9°C, a temperature commonly encountered by Gulf of Alaska Tanner crabs during fall, a prolonging of the degree-day intermolt duration occurred. The degree-day method of describing intermolt durations worked adequately at 3°-6°C, but above 6°C additional experiments are needed to better comprehend the influence of temperature on molting frequency. There was no obvious relationship between rearing temperature and growth. The average increase in carapace width for males held at 3°, 6°, and 9°C was 33% (SD = 4), 32% (SD = 6), and 32% (SD = 8) with no significant differences in growth per molt relative to rearing temperature detected.

Purpose of Project

In the northern Gulf of Alaska and the southeastern Bering Sea the Tanner crab, *Chionoecetes bairdi*, is a ubiquitous benthic invertebrate that is harvested commercially. The change in carapace size following molting has been described for Gulf of Alaska *C. bairdi* (Donaldson et al. 1981, Paul and Paul 1996) but the intermolt duration has not. Information on intermolt duration is critical to understanding recruitment to the population because there is no accepted method to age Tanner crabs (Rosenkranz et al. 1998). Crustacean growth rates are controlled by a number of factors with crab size and temperature being the most obvious (Fisher 1999). Benthic temperatures in the Gulf of Alaska experience long term warming and cooling cycles of about 18-20 years (Royer 1989). In addition to seasonal changes in temperature El Niño events are additional sources of thermal perturbations.

The objective of this study was to determine the intermolt period of juvenile and adolescent male Tanner crabs in degree days. In earlier observations of size of maturity and growth in male Tanner crabs (Adams and Paul 1983, Paul and Paul 1996) it appeared that immature males took less time to molt than mature males of similar sizes but this was not measurable with the methods used. We speculated that the molting schedules of mature and immature males would be markedly dissimilar, so we ascertained the maturity status of test specimens to examine this theory. We knew that temperature would modify intermolt durations, so we examined the intermolt durations of crabs of similar size but reared at different temperatures.

Approach

The Relationship Of Male Size And Maturity Status To Intermolt Durations

Male Tanner crabs were collected at the head of Resurrection Bay near Seward Alaska at 35-80 m depth using a 2 m otter trawl with 6 mm cod end mesh. There were 67 males with carapace widths (CW) 9-82 mm that were returned to the nearby laboratory for the study. The seawater for the Seward Laboratory comes from 75 m depth in a fjord and its temperature during the study was 3-10^{o}C. The temperature of the incoming water changes with season with marked interannual variations in monthly values. Each day the seawater temperature in the tanks was recorded. Salinity ranged from 31-33 ppt. All test animals were held in separate numbered tanks to prevent cannibalism. Males $\leq$ 45 mm were held in individual 20-L tanks. Males $\geq$ 46 mm were held in 100-L tanks and the water exchange rate in all tanks was 100% per h.

Captives were fed to excess every Monday (whole northern shrimp *Pandalus borealis*, Wednesday (live intertidal mussel *Mytilus trossulus*) and Friday (Coho salmon fillet *Oncorhynchus kisutch)*. Whenever a male molted for the first time in captivity the date of molting was recorded. After two weeks had passed, and the carapace had hardened, its maximum CW was measured to the nearest 0.1 mm. The new post molt CW of the smallest captive was 11 mm and the largest 105 mm CW. A 3 mm coded plastic disk was glued to the carapace of test specimens. The date of each crab's second molt was recorded to calculate the intermolt duration. The new CW was measured 2 weeks later and the vas deferens was removed to determine if spermatophores were present in wet mounts using 100x magnification. The intermolt duration was described in degree days. Degree days were calculated by summing the daily seawater temperatures that occurred during the intermolt period. For example if an event took ten days, and each day the temperature measurement was 10^{o}C, then the process would have taken 100 degree days.

Temperature effects on intermolt duration

Male Tanner crabs were captured with a small otter trawl at 20- to 100- m depths in Resurrection Bay and transferred to the laboratory. The rearing seawater came from 75-m depth, depths that juvenile Tanner crabs naturally inhabit. Individuals with carapace widths (CW) of <20 mm were held in tanks with flowing seawater. One week after molting the new CW was measured to the nearest 0.1 mm using a vernier caliper. If the new CW was 24-26 mm, its subsequent intermolt durations were calculated. Males of this size would be juveniles (Paul 1992).

Individual newly molted males (24-26 mm) were put into 12-L numbered perforated plastic tubs floating in 800-L tanks. The seawater exchange rate in the tanks was 10% per day. They were kept in near darkness (1 lux) except during feeding and cleaning periods. During their captivity the crabs were fed every Monday, Wednesday, and Friday with alternating meals of live intertidal mussel, tail meat and carapace from the northern shrimp and Coho salmon fillet. They were fed a surplus of food every time with excess food removed after 48 hours.

Test individuals were held in temperature regulated tanks at either 3^o (n = 13), 6^o (n = 12), or 9^oC (n = 12). The standard deviation for the mean three test temperatures was $\leq 0.5^oC$. One week after they molted, their new CW was measured to the nearest 0.1 mm and growth recorded as the percent increase in CW. Intermolt periods were described in days and degree days.

The ANOVA test and the Student-Newman-Keuls pairwise multiple comparison procedure was used to compare the intermolt durations for groups held at the 3 test temperatures. The ANOVA test was used to compare the percentage increase in CW for the 3 test groups after converting the percentages to arcsin values. All work was carried out by University of Alaska staff.

Results, Evaluation and Conclusions

The Relationship Of Male Size And Maturity Status To Intermolt Durations

The objective of relating intermolt duration in Tanner crabs to male size was achieved using the above methods. The relationship between male CW and intermolt duration exhibited a linear relationship for juveniles that was described by the equation: Intermolt period (degree days) = 7.9 (CW mm) + 272; r^2 = 0.53, P <0.0001, n = 53 (Fig. 1A). The relationship between CW (mm) and intermolt duration for males producing spermatophores was described by the equation: Intermolt period (degree days) = 7.6 (CW mm) + 1,215; r^2 = 0.14, P = 0.1832, n = 14 (Fig. 1B). The intermolt duration of mature males was less dependent on temperature than that of juveniles. The intermolt durations of males that were producing spermatophores (Fig. 1B, C) were longer than would be expected if the linear models of intermolt duration for juvenile individuals (Fig. 1A) and adolescents (Fig. 1B) coincided (Fig. 1C). For example, the largest male without spermatophores was 56 mm CW and it had an intermolt duration of 672 degree days (Fig. 1C). The smallest male with spermatophores was 55 mm CW and 1,692 degree days passed before it molted (Fig. 1C).

It is generally accepted that the amount of time that passes between molts increases with crab carapace size, and this proved to be true for *C. bairdi*. We are not sure why small spermatophore bearing males had longer intermolt periods than similar sized immature males. Perhaps spermatophore production is only one element of a complex maturation process with morphological, biochemical and physiological changes that involves longer intermolt periods. Currently, no other information on the intermolt durations of juvenile Tanner crabs, or closely related high latitude species, is available to compare to our results. In the laboratory a surplus of food was present while in nature food availability may limit growth rates. It is also possible that the laboratory diet was nutritionally incomplete. This study needs replication with *in situ* tagging studies to determine if our laboratory molting schedules are applicable to natural conditions.

These observations on intermolt durations demonstrate that size and maturity status are important factors to consider when forecasting molting schedules in Tanner crabs < 105 mm CW. Tanner crab males are not harvested until they are ≥ 140 mm CW and they can grow to ≈ 170 mm CW. Thus, molting rate studies need to be done with larger specimens than we used in this observation. Additional experimentation with large crabs would be especially important if there are maturation processes other than spermatophore production that influence molting schedules. Juvenile and adolescent males typically have relatively small claws while fully mature males have large claws (Stevens et al. 1993). All the males in this study were small claw morphotypes using the criteria of Stevens et al. (1993), the ratio of chela height/CW < 0.17, and most intermolt durations were one year or less. Males continue to molt after reaching maturity (Paul and Paul 1995) presumably because big large claw males win competitions for mates, and compromised carapaces need replacement. The large claw characteristic develops when males reach 100-130 mm CW (Stevens et al. 1993). We studied the consequence of spermatophore presence on molting rates, but not the change to the large claw morphotype, or the attainment of near maximum CW. In another investigation Tanner crabs with CW ≥ 110 mm had to be held for over 2 years before they molted (Paul and Paul 1995). None of them were soft-shelled when they were captured, so their intermolt period was longer than 2 years. In one *in situ* study 47% of tagged male *C. bairdi* > 110 mm CW were recaptured after 2 years and another 7% after three years (Donaldson 1980). These observations (Donaldson 1980, Paul and Paul 1995) suggest that intermolt durations increase after males assume the large claw morphotype and approach maximum size. Further growth rate studies with males 105-170 mm CW are needed to describe the intermolt durations of these large individuals.

Temperature Effects On Intermolt Duration

The objective of relating intermolt duration in Tanner crabs to water temperature was achieved using the approaches outlined in the original proposal. No test specimens died during the study, and at all 3 test temperatures captives fed voraciously. Increasing the rearing temperature from 3^o to 6^oC reduced the number of days between molts, but the trend did not continue when the holding temperature was set at 9^oC. The intermolt duration (Fig. 2A, B) for specimens held at 3^oC was 154 days (SD = 24) or 462 (SD = 74) degree days. At 6^oC comparable values were 78 days (SD = 14) or 467 (SD = 87) degree days. At 9^oC individuals molted after 74 days (SD = 8) or 665 (SD = 73) degree days. The number of days between molts for juvenile males held at 3^oC was significantly longer than the intermolt period in days for males at 6^o (ANOVA, Student-Newman-Keuls P<0.05) and 9^oC (P <0.05; Fig. 2A). There was no significant difference in the number of days between molts for the 6° and 9°C (P< 0.05) test groups (Fig. 2A). When the molting schedules were expressed in degree days the pattern of which test groups were statistically similar was reversed. At 3° and 6°C intermolt durations in units of degree days were not significantly different (ANOVA, Student-Newman-Keuls P<0.05) but at 9°C the degree day intermolt period was significantly longer than those observed at 3° (P <0.05) and 6°C (P<0.05; Fig 2B).

At 3°, 6° and 9°C CW increased by an average of 33% (SD = 4), 32% (SD = 6), and 32% (SD = 8) respectively (Fig. 2C). The ANOVA test indicated that there was no significant difference between the percent (arcsin) change in CW following the molt for groups held at the 3 temperatures (P = 0.855). Thus, the rearing temperature had little effect on carapace growth per molt of test specimens.

Typically increasing the temperature when rearing crabs reduces the intermolt period. However, the prolonged degree-day intermolt duration seen in Tanner crabs reared at 9°C (Fig. 2B) showed that at some point between 6° and 9°C increases in rearing temperature ceased to reduce the time between molts. At the capture site Tanner crabs would have encountered about 9°C during September-November. This is a nonlethal temperature but one that modifies molting frequency. Currently the upper thermal limits of Tanner crab have not been described. This experiment indicates that the degree day method of describing intermolt durations works well at 3°- 6°C but above 6°C additional experiments are necessary to improve our understanding of the effect of temperature on molting schedules.

In this study captives were well fed. Currently we have no information on the nutritional status of Tanner crabs in the wild, so these results should be used with caution when predicting *in situ* molting frequencies.

In addition to modifying the intermolt duration, the thermal conditions impact growth in some species. We did not observe conspicuous thermal related growth differences in juvenile Tanner crabs but that may be because we did not examine growth over a wider range of temperatures. The results of this study suggest that the effect of temperature on molting in Tanner crabs is complicated and caution must be used when predicting intermolt durations with the degree-day method.

Products

The results from this project will be published in two journal papers. The information on the effect of crab size on molting will appear in "Paul, A. J., and J. M. Paul. 2001. Intermolt durations of captive juvenile and adolescent male Tanner crabs *Chionoecetes bairdi*. Journal of Shellfish Research. *In press*". The material on the effect of temperature on molting duration will appear in "Paul, A. J., and J. M. Paul. Effects of temperature on length of intermolt periods in juvenile male Chionoecetes bairdi. Alaska Fishery Research Bulletin. *In press*".

KEY WORDS

Chionoecetes, molting, temperature

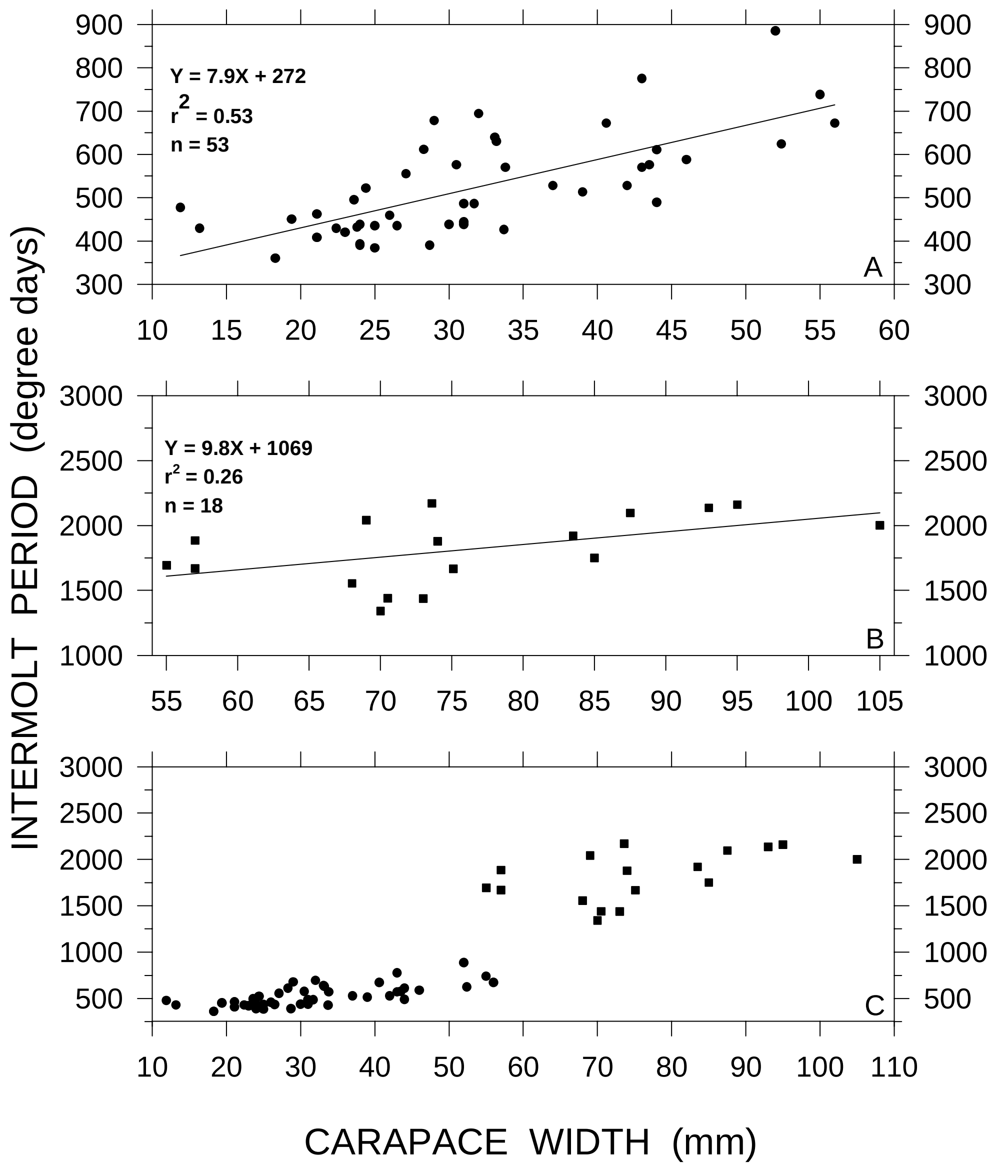

Figure 1. Intermolt durations in degrees days for juvenile male *Chionoecetes bairdi* that did not have spermatophores in their vas deferens (Panel A ●), spermatophore bearing males (Panel B ■), and comparisons of both types (Panel C).

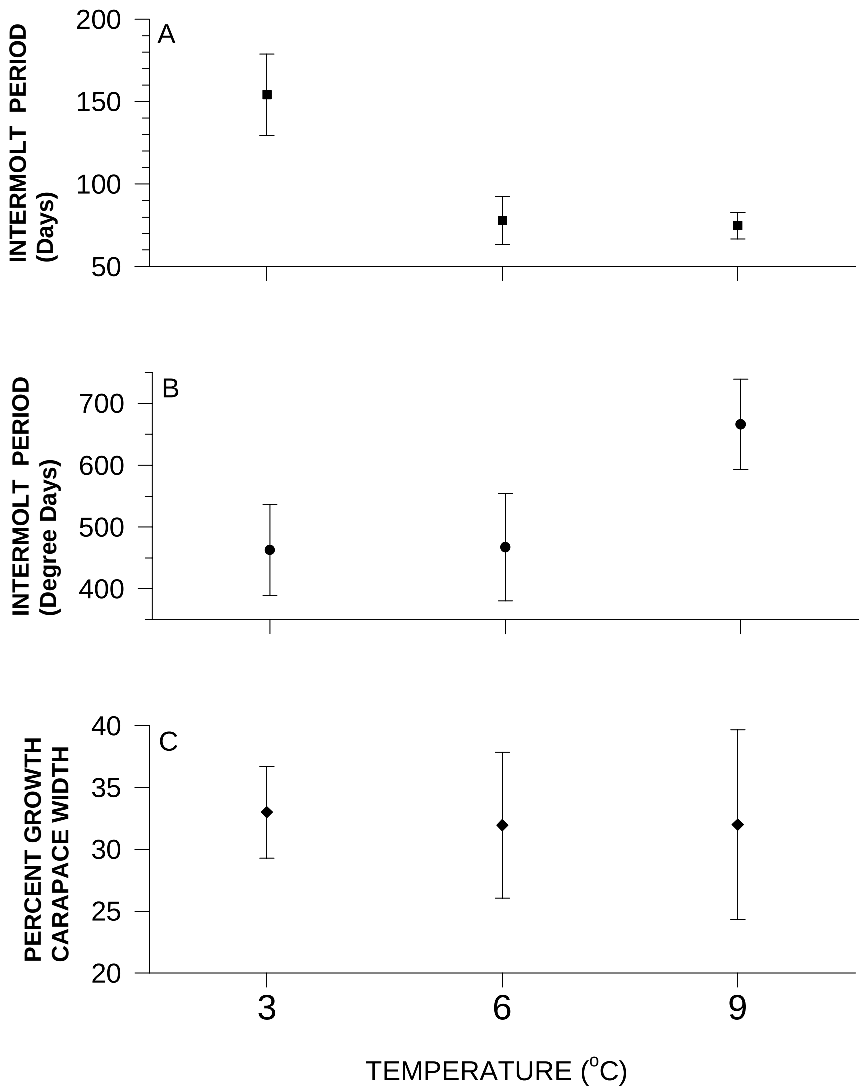

Figure 2. Intermolt duration of juvenile male Tanner crabs held at 3°, 6° and 9°C in units of days (A) and degree days (B). The percent increases in carapace widths of captives held at 3°, 6° and 9°C (C). Error bars are standard deviations.

PROJECT 6: THE REPRODUCTIVE CYCLE OF GOLDEN KING CRAB
Lithodes aequispinus

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Executive Summary

The golden king crab, *Lithodes aequispinus* Benedict*,* supports an important fishery in Alaska. This North Pacific species typically lives in deep water, on untrawlable bottoms. Because of their remote deep habitat, and the fact that only males are retained by the fishery, the reproductive cycle of females had not been described in detail prior to this study. Female golden king crab carry up to 27,000 large eggs (Jewett et al. 1985) that can hatch in any month of the year (Adams and Paul 1999). Female golden king crabs are found in all reproductive and molting stages throughout the year (Paul and Paul 2000a), so the duration between clutches was not apparent from gross examination of the egg mass.

During this project female golden king crabs *Lithodes aequispinus* were held in captivity to determine the duration of their reproductive cycle. The time between the first and last egg hatching was 34 days (SD = 16, n = 147) on average. Females molted about 192 days (SD = 72, n = 111) after the last egg hatched. Eggs were extruded 2 days (SD = 2, n = 61) after molting. Egg clutches were incubated for an average of 362 days (SD = 78, n = 59). The average amount of time that passed between the production of successive egg clutches was 590 days, or 3,570 degree days of water temperature.

Purpose of Project

This laboratory study examined the reproductive cycle of golden king crab to identify how long it took females to go from the extrusion of one egg clutch to another. This was the only objective of the project.

Approach

Crabs needed for the study were captured with pots fished at 108 to 152 m depth on the western side of Prince William Sound, Alaska. After capture the specimens were transported to the Seward Marine Center Laboratory by floatplane. No mortalities occurred during the transport process. Collections of multiparous females were made 11-14 November 1996, 1 May 1997 and 10-20 October 1998, and observations of captives continued until 1 October 2000.

The carapace length (CL) of all crabs was measured to the nearest mm for the growth study (Paul and Paul 2000a), and they were tagged with a numbered plastic disk attached to the leg with a plastic cable tie. All females in this study had egg clutches when captured and they had carapace lengths ranging from 104-150 mm (_ = 132, SD = 7mm). Each female was held in a separate 800-1000 L tank. The seawater in the tanks was exchanged ≥100% per hr to maintain the ambient temperature of incoming water from 75m depth in Resurrection Bay. The water temperature in the tanks was measured daily. The duration of each of the 4 reproductive phases was described as both number of days and degree days of water temperature. The degree day intermolt period is considered to be the sum of the average daily temperature during the intermolt period. Thus for example, if a stage interval was 30 days and the temperature every day was 6^{o}C, the event spanned 180 degree days (30 x 6). The degree day durations data were calculated because the reproductive phases were nonsynchronous with some females extruding new clutches in the warm part of the year and others during the cold season. Temperature in the tanks followed the fjord's seasonal cycles ranging from 3.7 to 9.7^{o}C. Crabs were fed every other day to excess with a repeating cycle of the following foods: whole Pacific herring *Clupea pallasi* Valenciennes 1847, fillet of coho salmon *Oncorhynchus kisutch* (Walbaum 1792), giant Pacific Octopus *Octopus dofleini* (Wulker 1910), whole squid (species unknown), and whole Alaska northern shrimp *Pandalus eous* Makarov 1995.

STAGE 1 (n = 147) was the length of time between the hatching of the first and last egg. STAGE 2 (n = 111) started after the last egg hatched and it ended when a female molted. STAGE 3 (n = 61) was the length of time between the day a female molted and the day she extruded a new egg clutch. The duration of clutch incubation (STAGE 4) was the length of time between the extrusion of a new egg clutch and the hatching of all those eggs (n = 59). Thus, STAGE 1 and 4 overlap. The durations of the different phases of the full reproductive cycle were not quantified for every female since captives had different collection dates.

Tanks were examined daily for the presence of larvae and when the first larvae were seen, females were examined every day until all eggs had hatched. This procedure was used to determine the duration of the larval hatching phase, STAGE 1. Thereafter, females were isolated from males until they molted. Females must molt prior to mating. These observations estimated the time between egg hatching and molting, STAGE 2.

After a female molted a selected hardshell male ≥114 mm CL was put into the tank with her. Males of this size are capable of fertilizing females (Paul and Paul 2000b). The time between the molt and the occurrence of the new egg clutch was termed STAGE 3. After ovulation, females were isolated and held until zygotes developed to the 64 cell stage. Then groups of at least 100 eggs from each pleopod were randomly selected and examined under a microscope for cell division to determine percent viability. Females were held from ovulation until hatching to determine the incubation period, STAGE 4.

This study was accompanied by parallel studies on growth of both sexes (Paul and Paul 2000a) and sizes at maturity of males (Paul and Paul 2000b).

Results, Evaluation and Conclusions

The single project objective, which was to describe the duration of the reproductive cycle of female golden king crab, was achieved and no modifications of methodological protocols were necessary during the study. All work was carried out by University of Alaska staff. We found that STAGE 1, the period between the first and last larvae hatching, averaged 34 days (SD = 16, n = 147, range 8-85) or 202 degree days (SD = 88, range 19-474). On average 192 days (SD = 72, n = 111, range 5-464) or 1,084 degree days (SD = 428, range 36-2,762) passed after the last larvae hatched until the females molted which completed STAGE 2. During STAGE 3 females typically extruded new eggs 2 days (SD = 2, n = 61, range 1-12) or 15 degree days (SD = 12, range 4-85) after molting. All clutches resulting from these laboratory matings had ≥80% of their eggs initiating division. STAGE 4, egg clutch brooding, lasted an average of 362 days (SD = 78, n = 59, range 40-569) or 2,269 degree days (SD = 570, range 114-2,754). The total time passing between egg clutches averaged 590 days or 3,570 degree days. The best fitting regressions relating the CL (mm) of all females that completed STAGES 1-4 and their reproductive cycle duration (degree days) showed no apparent relationship between these variables ($r^2 \leq 0.12$).

In early studies the time of spawning of golden king crab was described both as seasonal asynchronous and synchronous (Sloan 1985). Some of this reported variability may have been caused by an imperfect understanding of the reproductive cycle. During STAGE 2 (= 192 days) females carry decaying empty egg capsules on their setae (Sloan 1985) and this condition made it difficult for fishery observers to classify the reproductive status of females.

For Prince William Sound golden king crab, molting and hatching events can occur in any month in captive females (Paul and Paul 1999, 2000a). Thus, their reproductive cycle is markedly different from the genus *Paralithodes*. In red king crab *Paralithodes camtschaticus* (Tilesius) the female reproductive cycle is synchronous, lasting about one year (Paul and Paul 1990, 1997). In blue king crab (*Paralithodes platypus* Brandt) primiparous females may produce egg clutches annually and every two years for larger multiparous females. Like red king crabs, the eggs of primiparous and multiparous blue king crab hatch during the spring plankton bloom (Jensen and Armstrong 1989). Red king crabs hatch their eggs in spring so the larvae can feed on the plankton bloom (Paul et al. 1990), then all ripe females molt and breed soon after hatching is done. With golden king crab, hatching does not need to occur exclusively during the spring plankton bloom because their lecithotropic larvae do not feed and they can tolerate both summer and winter temperatures (Shirley and Zhou 1997, Adams and Paul 1999, Paul and Paul 1999). One striking difference between the reproductive cycle of golden and red king crabs is the amount of time between egg hatching and the female molt. In red king crabs captive females usually molt within 2 weeks of egg hatching (author's unpublished observations) vs. about 6 months for golden females. Currently it is not known why these differences exist. Golden king crab females may need more time to produce the large yolk rich eggs that allow their larvae to forgo feeding. However, that idea is speculation at this time.

Female Prince William Sound golden king crab first mature around 120 mm CL and typically grow to about 150 mm CL in 5 molts (Paul and Paul 2000a). If they produced an egg clutch every molt, and their reproductive cycles lasted 590 days, females would have to survive at least 8 years to produce 5 clutches.

The sea water for holding the crabs came from 75 m depth and during August to December it would have temperatures 1-4^{o}C warmer than ≈150 m where the specimens were captured vs. 1-2^{o}C cooler from January to April (Xiong and Royer 1984). Generally warmer conditions decrease intermolt durations in crustaceans unless there is thermally induced stress and cooler ones lengthen it. Only *in situ* studies, or laboratory studies that mimic site specific temperatures, can determine if our degree day estimates for the reproductive cycle duration are appropriate for female golden king crab living in different thermal environments.

The average amount of time that passed between the production of successive egg clutches was 590 days, or 3,570 degree days of water temperature. The reproductive cycle of female golden king crab is much longer than that of red king crab, or Tanner crab which produce new egg clutches annually.

Products

The results of this study were accepted for publication in the journal of shellfish research. The reference for this publications is: Paul, A. J. and J. M. Paul. 2001. The reproductive cycle of golden king crab *Lithodes aequispinus* (Anomura: Lithodidae). J. Shellfish Res. 20(1): In press.

Key Words

golden king crab, reproduction, reproductive cycle

PROJECT ADMINISTRATION

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Executive Summary

The grant, *King and Tanner Crab Research in Alaska*, was administered by Gordon H. Kruse. NOAA does not fund a specific project, called *Project Administration*, but some NOAA funds support travel and other marginal costs associated with grant administration by ADF&G. These funds provide the means for the project coordinator to discuss this federally funded crab research at meetings of professional fishery scientists, agency decision-makers, fishing industry, and environmental groups. The funds also foster annual interagency crab research meetings for coordinating ongoing research and developing future research plans.

Purpose of Project

The overall purpose of *Project Administration* is to ensure that all subprojects are: focused on high priority crab research and management needs, scientifically and technically sound, well coordinated with other state and federal projects and programs, achieve their intended objectives in a timely manner, and that their results are disseminated by publication in peer-reviewed scientific literature and agency reports and by presentations at meetings of scientists and interested public.

Approach

The ADF&G project coordinator, Gordon H. Kruse administered this contract for ADF&G, Division of Commercial Fisheries. *Project Administration* was not a formal project, but NOAA funds supported some of the costs associated with administration of this grant. Although the personnel costs of the project coordinator are paid by ADF&G with State of Alaska General Funds, this NOAA contract provided some funds to support the administration of this contract. In state fiscal year FY 00 (July 1, 1999 to June 30, 2000), NOAA supported $8,445, mostly for travel by the project coordinator, with some minor incidental costs associated contractual services (e.g., meeting room rental, registration fees, slide preparation) and supplies (e.g., film). For FY 01 (July 1, 2000 to June 30, 2001), NOAA supported $4,667, again mostly travel. The NOAA grant also funded $22,857 and $22,240 for administrative costs in FY 00 and FY 01, respectively, incurred by the ADF&G, Division of Administration.

The project leader was responsible for maintaining the scientific integrity of the component research projects, collaborating with some of the subproject investigators on some of the research, preparing semi-annual progress reports, and disseminating findings to the fishing industry, agency biologists, fishery managers, and scientific community.

Results, Evaluation and Conclusions

The administration of this grant has been successful, as evidenced by the success of the other projects and by the products listed below. During the award period, the project coordinator delivered eight oral presentations on this federally funded crab research at a range of meetings of professional fishery scientists, state and federal agency meetings, university seminars, and meetings with industry and environmental groups. During the award period, the project coordinator published six papers, 11 agency reports, and coauthored four manuscripts submitted for publication.

In December 1999, the project coordinator convened the annual interagency crab research meeting in Anchorage. The meeting was well attended by state, federal and university scientists. The meeting was a highly successful venue for the participants to discuss ongoing research, future research plans, and research coordination among scientists.

No meeting was held in December 2000, owing to the Lowell Wakefield Crab Symposium, held in January 2001. However, in association with the crab symposium, a one-day workshop was convened with a small group of crab scientists. The goal was to develop a comprehensive set of hypotheses on the physical and biotic factors that influence the variation in year-class success of snow crab *(Chionoecetes opilio)* stocks. A draft report is being finalized.

Overall, this grant, *King and Tanner Crab Research in Alaska*, has been highly successful. Grant administration has gone very smoothly, and NOAA grants officers are to be highly commended for their assistance in this effort and to contributing to the overall project successes.

Products

During the period funded by this grant, the ADF&G project coordinator delivered the following oral presentations on this federally funded crab research:

1. *Perspectives of Crab Stock Declines.* Presented to the Board of Directors, Alaska Marine Conservation Council, October 22, 1999, Juneau.

2. *Analysis of Minimum Size Limit for the Red King Crab Fishery in Bristol Bay, Alaska.* Presented at the American Fisheries Society, Alaska Chapter Annual Meeting, November 9-11, 1999, Kodiak, and the ADF&G seminar series, December 1, 1999, Juneau.

3. *Managing crab fisheries in a changing environment*. Presented at the Exxon Valdez Trustee Council Annual Restoration Meeting, January 18-19, 2000, Anchorage.

4. *Overview of Alaskan Crab and Scallop Fisheries*. Presented at the Alaska Region Workshop on Fishing Gear Impacts on Habitat, January 25, 2000, Auke Bay.

5. *Insights into Alaskan Crab Population Dynamics*. Presented at the School of Fisheries and Ocean Sciences, University of Alaska Fairbanks. January 28, 2000.

6. *A Rebuilding Plan for St. Matthew Island Blue King Crabs.* Presented to the Alaska Board of Fisheries, Anchorage, March 17, 2000, and the North Pacific Fishery Management Council, April 10-14, 2000.

7. *Climate Fluctuations and Dynamics of Alaskan Crab Populations*, Annual Meeting, PICES, October 20-28, 2000, Hakodate, Japan.

8. *Retrospective length-based analysis of Bristol Bay red king crabs: model evaluation and management implications.* Presented to the Lowell Wakefield CRAB2001Symposium, January 19, 2001, Anchorage.

Also, during this award period, the project coordinator published the following peer-reviewed publications and non-peer reviewed agency reports pertaining directly to work funded by this grant:

Peer-reviewed Papers

Kruse, G.H., L.C. Byrne, F.C. Funk, S.C. Matulich, and J. Zheng. 2000. Analysis of minimum size limit for the red king crab fishery in Bristol Bay, Alaska. North American Journal of Fisheries Management 20: 307-319.

Rosenkranz, G.E., A.V. Tyler, and G.H. Kruse. 2001. Effects of water temperature and wind on recruitment of Tanner crabs in Bristol Bay, Alaska. Fisheries Oceanography 10: 1-12.

Zheng. J., and G.H. Kruse. 2000. Rebuilding probabilities under alternative rebuilding strategies for eastern Bering Sea Tanner crab. Alaska Fishery Research Bulletin 7: 1-10.

Zheng. J., and G.H. Kruse. 2000. Recruitment patterns of Alaskan crabs and relationships to decadal shifts in climate and physical oceanography. ICES Journal of Marine Science 57: 438-451.

Zhou, S., and G.H. Kruse. 2000. Modifications of cod pots to reduce Tanner crab bycatch. North American Journal of Fisheries Management 20: 897-907.

Zhou, S., and G.H. Kruse. 2000. Capture efficiency and size selectivity of two types of pots for red king crabs in the Bering Sea. Alaska Fishery Research Bulletin 6(2): 94–103.

Agency Reports:

Kruse, G.H., F.C. Funk, H.J. Geiger, K.R. Mabry, H.M. Savikko, and S.M. Siddeek. 2000. Overview of state-managed marine fisheries in the central and western Gulf of Alaska, Aleutian Islands, and southeastern Bering Sea, with reference to Steller sea lions. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J00-10, Juneau.

Kruse, G.H., editor**.** 1999. King and Tanner crab research in Alaska: annual report for July 1, 1998 through June 30, 1999. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J99-09, Juneau.

Kruse, G.H., editor. 2000. King and Tanner crab research in Alaska: a semi-annual report for July 1, 1999 to December 31, 1999. Alaska Department of Fish and Game, Division of Commercial Fisheries, Unpublished report, Juneau.

Kruse, G.H., editor. 2000. King and Tanner crab research in Alaska: a semi-annual report for January 1, 2000 to July 31, 2000. Alaska Department of Fish and Game, Division of Commercial Fisheries, Unpublished report, Juneau.

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Kruse, G.H., editor. 2001. King and Tanner crab research in Alaska: a semi-annual report for January 1, 2001 to July 31, 2001. Alaska Department of Fish and Game, Division of Commercial Fisheries, Unpublished report, Juneau.

Murphy, M.C., and G.H. Kruse. 1999. Federal requirements for State of Alaska management measures under the auspices of the fishery management plan for Bering Sea/Aleutian Islands king and Tanner crabs: a report to the Alaska Board of Fisheries. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J99-03, Juneau.

Zheng, J., and G.H. Kruse. 2000. Status of king crab stocks in the eastern Bering Sea in 2000. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J00-09, Juneau.

Zheng, J., and G.H. Kruse. 2000. Overview of stock assessment and recommended harvest strategy for St. Matthew Island blue king crabs. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J00-06, Juneau.

Zheng, J., and G.H. Kruse. 1999. Overview of population dynamics and recommended harvest strategy for Tanner crabs in the eastern Bering Sea. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J99-04, Juneau.

Zheng, J., and G.H. Kruse. 1999. Status of king crab stocks in the eastern Bering Sea in 1999. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J99-09, Juneau.

The project coordinator coauthored the following submitted manuscripts, funded in part by this grant, during the award period:

Zheng, J., and G.H. Kruse. MS. Retrospective length-based analysis of Bristol Bay red king crabs: model evaluation and management implications. Submitted to Lowell Wakefield Crab 2001 Symposium, University of Alaska Sea Grant College Program, submitted.

Zheng, J., and G.H. Kruse. MS. Massive die-off or change in catchability: implications on stock assessment and fishery management. Submitted to Lowell Wakefield Crab 2001 Symposium, submitted.

Kruse, G.H., N. Bez, A. Booth, M. Dorn, S. Hills, R. Lipcius, D. Pelletier, C. Roy, S. Smith, and D. Witherell (editors). 2001. Spatial Processes and Management of Marine Populations, University of Alaska Sea Grant College Program, Report AK-SG-00-04, Fairbanks, *in press*.

Zheng, J., and G.H. Kruse. *In press*. Spatial distribution and recruitment processes of snow crabs in the eastern Bering Sea. *In* proceedings of the International Symposium on Spatial Processes and Fisheries Management, University of Alaska Sea Grant College Program, Report AK-SG-00-04, Fairbanks.

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